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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 47 JANUARY 1, 1919 No. 4

STUDIES ON THE SUBMAXILLARY GLAND

I. Electrical Deflections in General[1]

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Received for publication October 26, 1918

GENERAL AND INTRODUCTORY

The object of this research is to gain more data on the significance of electrical deflections[2] of living tissue.

The work here reported represents data obtained from experiments on the submaxillary gland of the dog. The choice of the submaxillary gland for an object of study is obvious. In the first place, it is a small compact mass of tissue lying near the surface and easily dissected. It has two sets of nerves differing considerably in function, likewise easily accessible. The volume-flow of blood may be controlled mechanically and by nerve stimulation and this flow measured. Electrodes can be easily and more or less uniformly applied. The work of the gland may be quantitatively controlled and measured. These advantages have appealed to many for study of phenomena in living tissue and consequently there is a very considerable literature on the physiology of the salivary glands at our disposal. This is of no small importance as regards the interpretation of such a variable factor as electrical deflections.

[1] Reported before the American Physiological Society 1916 and 1917. Proceedings in this Journal, 1917, xlii, 591 and 1918, xlv, 559.

[2] The term electrical deflection is preferred to that of electrical variation. The term electrical variation will be employed only to describe variations in electrical deflections.

In reading the literature[3] on electrical physiology of glandular structures one is impressed with the variability of deflections obtained even when the tissues presumably are activated and treated in the same manner. The difficulty of explaining these variations, particularly the complete reversal of deflections, has been encountered by many. Unfortunately in the study of electrical physiology of glandular structures the graphic method has been little employed. Visual observations of the deflections of the galvanometer sufficed to supply the data. A trial of this method demonstrated the difficulty of gaining a clear picture of the electrical deflections, let alone the time relation of synchronous phenomena in the gland. Therefore it seemed important to use a graphic and continuous method with which every deflection in the course of an experiment can be recorded. The use of the photographic method with the string galvanometer as employed by Schaeffer, and with the d'Arsonval galvanometer as developed by Cannon, were considered but the synchronous photographing of chorda stimulation, of sympathetic stimulation, of secretion, volume-flow of blood and electrical deflections seemed too cumbersome and difficult. In addition, if a large number of experiments are performed the method becomes expensive, but most important is the inability to follow the progress of the experiment. A picture of the results is available only after the experiment is completed. The importance of knowing at the time when constant deflections are obtained so that a new variable might be introduced became evident in the course of the research and proved to be a most important factor.

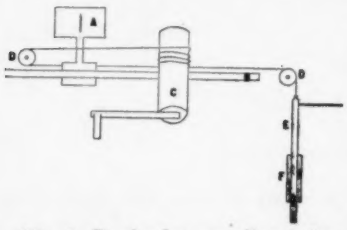

Fig. 1. Device for recording deflections of a d'Arsonval galvanometer on smoked paper. *A*, sliding target for following the reflected filament; *B*, guide for sliding target; *C*, windlass operating the target; *D*, pulleys; *E*, weighted shaft carrying writing point; *F*, guide for weighted shaft.

A graphic method of recording deflections of a d'Arsonval galvanometer on smoked paper was developed. With this method the employment of controls was an easy matter. While using the method I learned that Spaeth employed the same principle for recording the movements of melanophores, and more recently, that the method has been in use in psychological laboratories for recording electrical deflec-

[3] The review of literature is reserved for later papers.

tions. But the device developed proved of such value in this research that it is shown in figure 1. The deflections are followed by an assistant. A horizontally sliding target, operated by a windlass, is kept in line with the horizontally reflected filament of the galvanometer. Attached to the target by thread is a vertically moving writing point which records the deflections. With the type of galvanometer[4] used the deflections were easily followed. Though at times these deflections are rapid and require some skill to follow promptly, the lag is only momentary and imperceptible with the slowly revolving drum usually employed.

By the use of this method, employed continuously throughout an experiment, a partial explanation of the variability of deflections was made possible. That some of these variations are more apparent than real is shown.

The Williams box proved most convenient for controlling the compensating current. The non-polarizable electrodes were the usual cotton, filter paper variety.

The electrical deflections accompanying glandular activation and recovery were continuously recorded. The gland was most commonly activated by stimulation of the chorda tympani, by stimulation of the sympathetic fibers alone or with the vagus fibers, and by injection of pilocarpin. The effects of adrenin, pituitrin, atropin, saline solution, etc., and of mechanical interference of the blood supply to the gland or interference to the flow of saliva through the duct were also determined.

To assist in the interpretation of the accompanying deflections, various synchronous records were made: blood pressure, volume-flow of blood by an automatic and bloodless method, salivary secretion, time in seconds, time and duration of stimulation, of injections, of change in blood supply and of obstruction of the salivary duct.

Two types of leads were employed the "two gland lead" and the "one gland lead." With the two gland lead both glands are exposed and an electrode placed on the outer surface of each. This arrangement suffices for studying effects of procedures limited to one gland only, such as stimulation of the glandular nerves on one side or obstruction of the salivary duct. If the effects of activation are bilateral, as with injections, isolation of one gland by a previous mass ligature is

[4] The galvanometer used in this research is made by the Leeds & Northrup Company. They give the following description in bulletin 228.—Galvanometer Resistance Ohms 550, Sensitivity 10,000 megohms = 10^{-10} amperes, Period Seconds 17.5, Critical damping resistance, external, ohms, 20,000.

Fig. 2. An electrical deflection obtained with prolonged chorda stimulation, showing the four crests and four depressions, the four ascending limbs or negative components and the four descending limbs or positive components. *B.P.*, blood pressure; *S*, salivary secretion; *T*, time in seconds; *E*, electrical deflection. The entire deflection required about eighteen minutes for its completion.

necessary. Such a ligature initiates changes in the gland, disturbing the electrical equilibrium for some time. But if this ligature is made at the beginning of the operation equilibrium is usually established by the time observations of electrical deflections begin.

When the "one gland lead" is employed both electrodes are placed on the activated gland, one usually on the hilus and the other on the outer surface opposite the hilus or a point slightly posterior to it.

With the "two gland lead" the electrodes are always placed so that an upward deflection indicates an increased negativity of the lead on the activated gland (the active lead) with respect to the lead on the resting or dead gland (the indifferent lead). With the one gland lead the hilus is usually the constant lead (exceptions to this will be noted). Upward deflection indicates the outer surface of the gland to be electrically negative to the hilus surface.

Each type of lead gives a more or less characteristic deflection—sometimes very similar, depending upon the points of the gland led from. But even though the general appearance of the deflections be dissimilar, careful study of the crests and depressions will show them to be comparable.

A typical deflection, with the fundamental crests and depressions, obtained by chorda stimulation of about sixty seconds duration with a "two gland lead," is shown in figure 2. It is composed of four negative waves or crests, 1, 2, 3 and 4, and four depressions, 1, 2, 3 and 4. The upstrokes are designated as negative components 1, 2, 3 and 4, and the down strokes as positive components 1, 2, 3 and 4.

At the beginning of this research the "two gland lead" alone was employed and with it a degree of similarity of deflections was obtained which gave promise of approaching a uniformity of results necessary for interpretation of the deflections, yet not infrequently marked dissimilarities occurred. Therefore the problem was to determine the nature of these unknown variables in order that only one new and known variable at a time might be introduced for study.

The maintenance of the uniform condition of the animal, a uniform application of the leading off electrodes and the uniform application of the stimulating electrodes proved very important in reducing the variable factors. This is evident, for a single deflection may last as long as twenty minutes and such deflections separated by an hour or more are frequently compared.

In addition to the variability of the individual deflections we often have a continual shifting of the electrical base line, the electrical con-

dition of rest. This shifting of the base line has a double source. As in the case of the variability of deflections, it also is dependent upon uniformity in the condition of the tissues and in the application of the leading off electrodes.

The most important factor in keeping the condition of the animal constant is uniform anaesthesia which was accomplished by the administration of a relatively large injection of morphine sulphate. The amount of ether given was usually adjusted at the beginning of the experiment and kept at that adjustment to the end for it was noted that a sudden change in the etherization often produced an electrical deflection.

The other source of change in the base line is at the leading-off electrodes—attributable to either shifting of position or to drying. A considerable shift in the position of the gland is without effect on the base line provided the wisp of cotton of the electrode moves with the gland. Electrodes mounted on rods of block tin and properly applied, together with sufficient anaesthetization to prevent the larger movements overcome this difficulty. A common source of shifting of the base line is the drying of the electrodes but contacts consisting of heavy wisps of cotton, frequently moistened with saline prevent such shifting.

Uniformity of application of the stimulating electrodes is absolutely necessary in quantitative work and for comparison of a series of deflections with long intervening periods. By employing certain precautions uniformity of stimulation is obtainable. The chorda lingual nerve, dissected to the maximum length, with all the branches cut excepting the chorda tympani, was placed in shielded electrodes mounted on rods of block tin and protected by moist cotton. Injury to the chorda tympani fibers was prevented and diffuse stimulation secured by placing the thickest part of the nerve in the electrodes and by using large animals. The cut end of the nerve was weighted with thread and lead weight so as to maintain constant pressure against the electrodes.

Presumably any change in the metabolic processes of a tissue is accompanied by some electrical response. The factors which may influence the processes of the submaxillary gland are therefore very numerous. Strength of stimulation of the nerves leading to the gland, duration of stimulation, period of rest between periods of stimulation, variations in the volume-flow of blood, resistance to the flow of saliva, injection of pilocarpin, adrenin, atropin, etc., are among those studied.

In this paper only data of general interest are considered. A more detailed study forms the basis of another paper.

Activation of the gland by stimulation of the chorda tympani is the most perfectly controllable means of altering the processes in the gland. It is the only method used in the expreriments described in this paper which deals with the effects of, *a*, duration of stimulation; *b*, strength of stimulation; *c*, period of rest between periods of stimulation; *d*, exercise, and *e*, leads.

In studying the effect of varying any one of these factors it is necessary to know that the particular change introduced is the only, or at least the main, factor varied. This can be the case only if there are

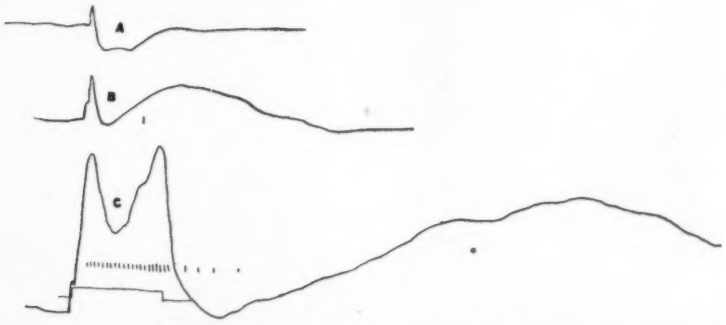

Fig. 3. Deflection showing the effect of varying the duration of stimulation. Secretion in drops is shown in curves *B* and *C*. In curve *A*, the stimulus was too short to elicit secretion. Crest number four is only partially shown in figure 3, *C*.

no slowly progressing changes in the animal as a whole or in the gland itself as a result of activation. It is therefore necessary to make control observations to determine whether any unknown variable is operating. In making these controls conditions were kept as constant as the methods permitted, i.e., a constant lead was employed, the chorda lingual nerve stimulated the same length of time, with the same strength of stimulus and with equal periods of rest intervening. If similar deflections were obtained with similar activation as indicated by the number of drops of saliva and the electrical deflection, it was assumed that conditions were constant enough for introducing a variable. Very frequently at the beginning of an experiment the gland reacted differently for a period of an hour or more, but occasionally equilibrium

of the base line and uniformity of deflection were established in a short time.

a. Duration of stimulation. By keeping constant the lead, the strength of stimulation and the period of rest between periods of stimulation, the effect of varying the duration of stimulation was studied (see fig. 3). The lead in this case was a "one gland lead," one electrode resting on the hilus of the activated gland and the other approximately opposite this point on the outer side of the gland.

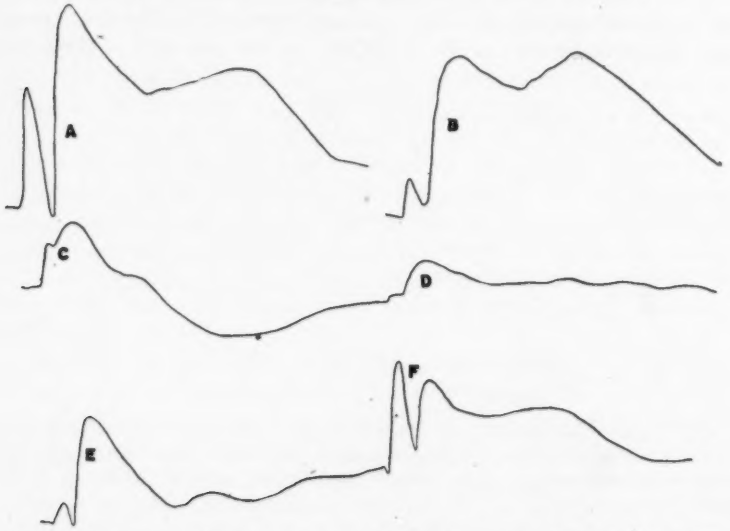

Fig. 4. Deflections showing the effect of changing strength of stimulus. The lead is constant, the period of rest five minutes, the period of stimulation twenty seconds. The strength of stimulation is progressively decreased from *A* to *D* and again increased from *D* to *F*.

With a very short stimulus of about a second's duration, crest number 1 alone may appear as a low sharp wave. Frequently this is followed by a low slowly developing wave, which may be crest number 4 (see fig. 3, *A*). By prolonging the stimulation, crest number two is added and the appearance of crest number 4 made more certain (fig. 3, *B*). With the addition of crest number 2, crest number 1 may not always be as distinctly seen. This depends on the rapidity of the development of the second negative component. If this component

develops strongly before the first negative component has completed its effect upon the galvanometer and before the first positive component comes into play, the first and second negative components may be more or less completely fused. In certain experiments the second negative component may develop so slowly that the first positive component reaches the base line and separates crests 1 and 2 completely. In figure 3 fusion is only partial. Though many curves show complete

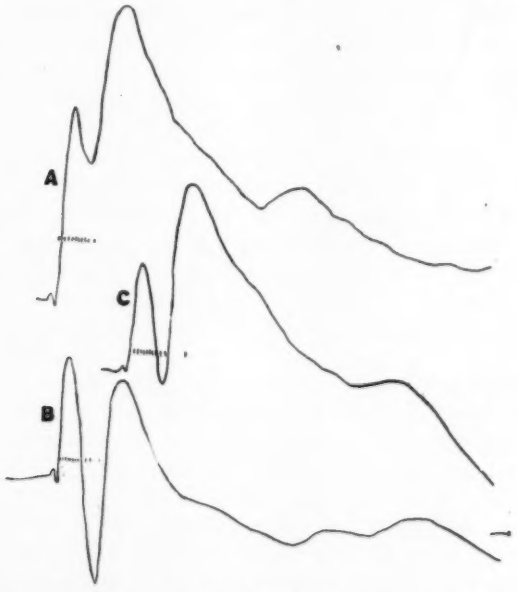

Fig. 5. Deflections showing the effects of rest. Strength of stimulation was constant, the duration of stimulation was twenty seconds in each observation. The period of rest preceding deflection *A* was 30 minutes; *B*, 5 minutes and *C*, 15 minutes. Though the same amount of secretion is elicited in each case the deflections vary but approximate each other with the approximation of the period of rest.

fusion component number 1 can be elicited by special methods in every animal. If the stimulus is prolonged still more than in 3 *A* and *B*, crest number 3 is added and crest number 4 is usually enlarged (fig. 3, *C*). This is the common picture produced by prolonged chorda stimulation; the original is shown in figure 2. There are, however,

certain exceptions—additional waves may appear and crest number 4 may be absent. These will be considered in a later paper.

b. Strength of stimulus. By keeping constant the duration of stimulation, the duration of rest and the lead, the effect of varying the strength of stimulation may be studied, as shown in figure 4. In these

Fig. 6. Observations showing a changing secretory response to stimuli of equal strength and duration applied at equal intervals, with the development of reversal of the electrical deflection.

observations the strength of stimulation is progressively diminished from *A* to *D* and increased again to *F*. A changing contour with diminishing strength of stimulation and the approximate duplication of curves on increasing the strength of stimulation, such as is seen here, occurs even though two instead of one variable may be introduced in this experiment. The difficulty of confining the variables to strength

of stimulation alone is apparent, for a period of rest following a period of strong stimulation is relatively shorter than a period of rest following a weaker stimulation. In these observations, however, strength of stimulation appears to be the important variable.

c. Duration of rest. The effect of rest is shown in figure 5, three observations in which the lead, the duration and strength of stimulation were constant but the rest preceding such stimulations variable. The period of rest preceding observation *A* was thirty minutes; observation *B* five minutes; observation *C* fifteen minutes. The change in contour with changing rest and the approximation of contour with approximation of the period of rest become obvious upon comparing deflections *A*, *B* and *C*. This change in contour is particularly interesting in view of the fact that the amount and rate of secretion remain the same. In the two preceding experiments on the effect of duration and of strength of stimulation, the contour of the deflections may be largely accounted for by the changing rate and amount of secretion. These factors are however missing in the present instance. The difference in the deflections accompanying equal secretions may possibly be looked upon as an effect of altered time relation between such processes as liberation and elaboration of secretion resulting from altered periods of rest.

d. Exercise. Occasionally, as in the preceding experiment, a previous stimulation of the chorda tympani has little or no noticeable effect upon the amount of secretion obtained by subsequent stimulation. Not infrequently, however, when a series of stimuli, of equal strength and duration with equal periods of rest, is applied to the chorda lingual nerve the preceding stimulus has its effect on the subsequent stimulus. This has been looked upon as an effect of exercise. When the electrical deflections have been recorded in such observations changes in contour occurred. (See fig. 6.) The general contour of the curves varies considerably, producing what is known as a reversal of the deflection, but a more careful study shows that these variations are more apparent than real—that the fundamental crests appear in each deflection and that it is only their amplitude and positions which are altered by a change in the balance of the adjacent negative and positive components. To what extent these changes in balance are due to the effect of exercise and how much to the increased response of the gland to stimulation, it is difficult to say. But the observation is of interest in that it shows a difference in response to stimulation and also that it shows the gradual development of a so-called reversal.

The observation illustrates the difficulty occasionally met with in interpreting deflections and in securing proper controls.

e. Lead. The two general types of leads—"one and two gland leads"—have already been described, and mention has been made of the fact that the deflections obtained with these two leads may resemble each other closely. To facilitate the description of various leads, certain points on the gland are numbered from 1 to 8. (See fig. 7). Numbers 1, 2 and 3, are on the outer side of the gland, 4, 5, 6, 7 and 8 on the edge of the gland between the outer and inner surfaces. The points corresponding to 1, 2 and 3 on the inner surface of the gland are numbered 1′, 2′ and 3′; 2′ corresponds to the hilus of the gland. If the lead is a "one gland lead," the leads coming from the hilus and a point directly opposite on the outer surface, it is designated lead 2′ – 2. Occasionally the lead may not be over a numbered point; e.g., it may be between 2 and 3. In that case the lead is designated as lead 2′–(2–3).

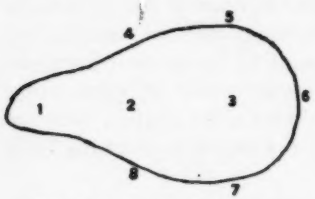

Fig. 7. Schema of the submaxillary gland showing leads.

If the lead is a "two gland" or a symmetrical lead, one electrode (the indifferent electrode) on the dead or resting gland and the other, e.g., on point 2 of the activated gland, the lead is designated as lead S – 2.

In figure 8 is shown a series of deflections obtained with a two gland lead resulting from chorda stimulations of equal duration (10 seconds) and equal intensity, the points of application of the electrodes being designated for each deflection. The deflection changes distinctly with each lead, more with some than with others, e.g., in observations *A*, *B* and *C* (leads S – 2, S – 1, and S – 2′), the electrical variation is greater than in observations *C*, *D* and G (leads S – 4, S – 5 and S – (5 – 6)). Each deflection is typical for the lead for when pairs of observations are taken for each lead the deflections in each pair show a close resemblance to each other, which is absent in deflections from different leads. Or if one is fortunate enough to keep conditions very constant, with careful application of the electrodes, duplicate series may be produced. In figure 8, deflections *A* and *F*, and *C* and *G* (each pair having approximately the same lead) resemble each other most closely even though separated by a considerable interval of time. (With observations *A* and *F* the leads presumably are the same; with observations *C* and *G*, the leads are only approximately the same).

Figure 9 shows a series of deflections obtained with a "one gland lead." The typical difference between a one and two gland lead is brought out fairly well by comparison of figures 8 and 9. In the series of figure 9, even though the lead was changed for each subsequent observation, there is an approximate duplication of the deflec-

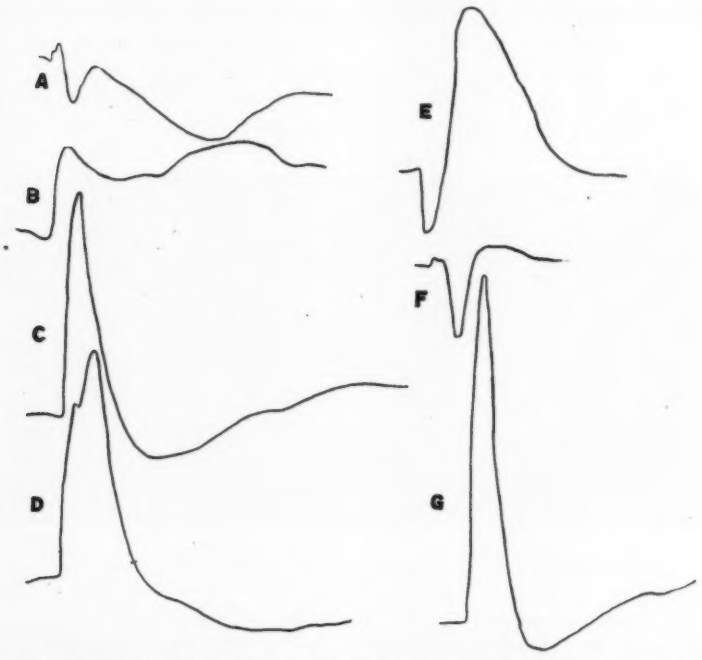

Fig. 8. Deflections showing the effect of changing leads. The curves are obtained from "two gland leads, "the lead on the dead gland remains constant, the lead on the activated gland is shifted as designated in the figure. *A*, S – 2, *B*, S – 1; *C*, S – 5.5; *D*, S – 4; *E*, S – 2′; *F*, S – 2; *G*, S – 5.

tions when the leads are repeated as in observations *B* and *H*. (Leads 2′ – 1), *E* and *F* (leads 2′ – 3), and *D* and *G* (leads 2′ – (3′ – 6)).

The observations represented in figures 8 and 9 illustrate the importance of knowing definitely the lead employed if an interpretation of the deflection is to be made or the observations of different workers are to be compared. Though in the "two gland leads" the deflections

are mainly above the electrical base line of rest, in the "one gland leads," large deflections below the base line occur. The extent to which this occurs in the "one gland" lead differs with the points led off. For example, deflection *B*, when compared with deflection *A* (fig. 9), might be considered a complete reversal yet the processes represented by the deflections are the same. Therefore a description of a reversal of the usual deflection without mention of the lead means

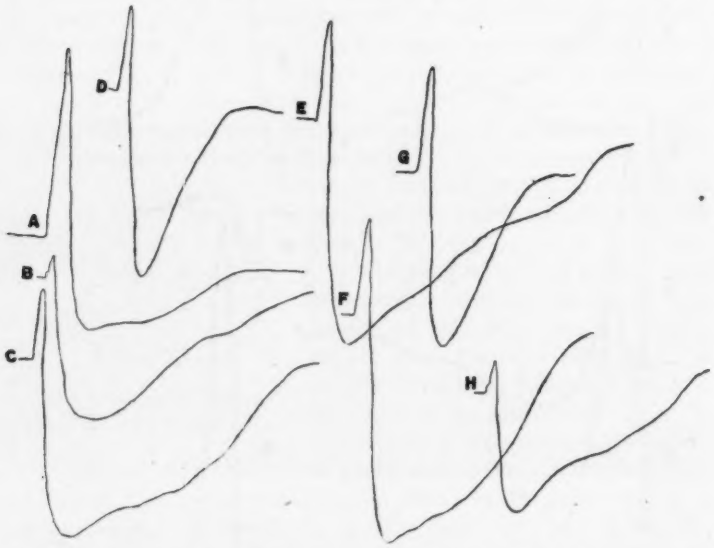

Fig. 9. Deflections showing the effect of changing leads. The curves are all obtained from "one gland leads," the 2′ or hilus lead remaining constant. *A*; 2′–6; *B*, 2′ – 1; *C*, 2′ – 2; *D*, 2′ – (3′ – 6); *E*, 2′ – 3; *F*, 2′ – 3; *G*, 2′ – (3′ – 6), *H*, 2′ – 1.

little. The reversal with a constant lead as seen in figure 6 however, is of interest.

f. Duration of stimulation, strength of stimulation, period of rest and leads constant. From the observations so far cited it is apparent that the electrical deflections are attributable to the nature and the sequence of processes going on within the gland and to the type of lead employed to register the electrical changes accompanying these processes. That comparable control deflections can be obtained was pointed out in the

beginning of the paper and now that the effect of varying a number of factors has been shown it might be of interest to know how constant the conditions in a gland may be kept and how accurately these conditions are reflected in the electrical deflections.

If we have an animal whose condition remains constant and if we employ a series of stimuli of equal strength and duration separated by equal periods of rest, the nature and sequence of the secretory and recovery processes should be the same provided the period of rest is long enough for recovery and the effects of exercise do not enter. If these conditions are met one might expect the electrical deflections to be constant with a constant lead. It is only if this is true that the

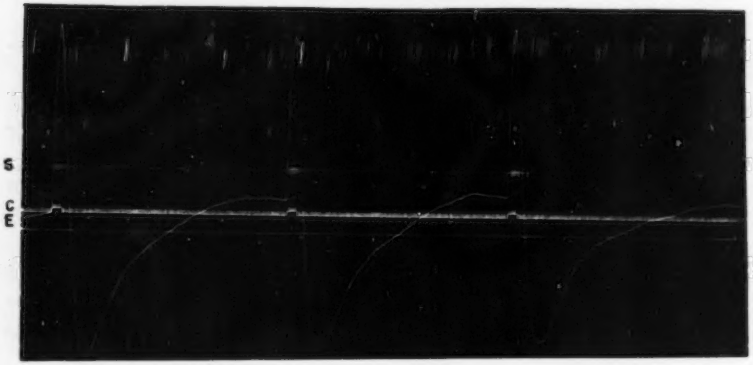

Fig. 10. Deflections obtained under constant conditions with strength and duration of stimulation and the intervening periods of rest equal.

electrical deflections may be considered a reliable index to glandular processes and that interpretations of the deflection become possible.

In the series of observations shown in figure 10 the chorda tympani nerve was stimulated in each case for 8 seconds with stimuli of equal strength, a constant period of rest of 5 minutes intervening. In each case exactly eight drops of saliva were secreted. The deflections are practically superimposable and reflect the constancy of intensity and sequence of glandular processes.

Knowing in a general way the significance of some of the more important variables we are better prepared to study in detail certain factors influencing glandular function and, therefore, the electrical responses.

It will be noted in figure 2 that secretory fluctuations run parallel with crests 2 and 3. To study the relation of these and other phenomena to blood-flow an automatic and bloodless method of recording the volume-flow of blood was devised and is described in paper II. In figure 3 of that paper a record of volume-flow of blood with prolonged chorda stimulation is shown. Paper III deals with the nature of the fluctuation in flow there noted, with the effect of volume-flow of blood on metabolism, the effect of metabolism on volume-flow of blood.

SUMMARY

The object of this research was to study the significance of electrical deflections in living tissue.

The submaxillary gland was chosen as a type of tissue easily accessible for study and with functions well controllable.

The gland was activated by stimulation of the chorda tympani fibers, under varying circumstances, with stimuli of varying intensity and duration. The associated deflections were recorded.

On account of the great variability of electrical deflections common to glandular structures, a continuous and graphic method of recording these deflections on smoked paper was employed.

The advantages of this method over the photographic method are discussed. It permits the recording of every deflection, the establishment of control observations and shows the results during the progress of the experiment.

To aid in the study of these deflections, blood pressure, salivary secretion, volume-flow of blood and time in seconds were synchronously recorded.

A prolonged chorda stimulation of about sixty seconds duration usually produces a deflection of four crests, the result of a balance of four negative and four positive components.

Though most deflections are comparable in their essential points, employing a single type of lead and activating the gland presumably under constant conditions elicits deflections varying in general contour.

To determine the unknown variables producing these electrical variations special precautions were taken.

By keeping the general condition of the animal constant, using a constant lead and activating the gland with stimuli of equal duration, equal intensity and separated by equal periods of rest, it was possible to obtain superimposable deflections.

With the aid of such controls it was shown, by varying single factors, that the contour of the electrical deflections may be decidedly altered by change of strength of stimulation, of intensity of stimulation, of leads, of the period of rest and by the factor of exercise.

Where the leads are constant the electrical variations may be attributable to changes in intensity and sequence of such processes as liberation of secretion, elaboration of secretion, recovery, etc.

The cardinal points of a typical deflection are usually present, though the balance of components may change markedly the general contour of the deflection.

This contour may change to what is known as a reversal. If the change in deflection occurs with a constant lead it is of significance. If it occurs as result of an altered lead it is of no value in indicating altered glandular processes.

This research is considered only as a necessary preliminary to further detailed study.

STUDIES ON THE SUBMAXILLARY GLAND

II. An Automatic and Bloodless Method of Recording the Volume-flow of Blood[1]

ROBERT GESELL

From the Physiological Laboratory of Washington University School of Medicine, St. Louis

Received for publication October 26, 1918

In the study of electrical variations of the submaxillary gland, I found that an interpretation of these variations required a continuous record of the volume-flow of blood through the gland.

Though the electrical variations are purely local reactions, they nevertheless are influenced by fluctuations of the general condition of the animal and for that reason it is essential to keep this condition as uniform as possible. Since at times it is necessary to compare deflections separated by intervals of an hour or more, the usual methods of recording the volume-flow of blood would introduce serious difficulties. The rigid requirements of the experiment suggested the importance of developing a new method for recording the flow of blood.

In the method which was developed the blood, without coming in contact with any foreign substance, is automatically measured as it flows, at approximately zero pressure, through the vein toward the heart. (See figs. 1 and 2.)

In measuring the volume-flow of blood of the submaxillary gland, *1*, the jugular vein, *2*, is cleanly dissected. Every branch with the exception of the branches coming from the gland is ligated. The vein is then placed in the trough, *3*, of the instrument under the emptying-plate, *4*, and under the cut-off, *5*, which rests upon an extension of the floor of the trough. The emptying-plate and cut-off are pivoted at *6* and *7* and are operated respectively by the emptying solenoid, *8*, and the cut-off solenoid *9*. The cut-off is held down on the vein by

[1] Reported before the American Physiological Society. Proceedings, this Journal, 1918, xlv, 545.

the constant pull of the spring, *10*, attached to the frame of the cut-off solenoid. The emptying-plate is perfectly balanced and free to swing in a vertical direction.

This arrangement permits the automatic filling and emptying of the vein lying in the trough. With the vein empty and the cut-off closed, the vein is filled by the blood coming from the gland and raises the emptying-plate until the cut-off contact, *11*, is made. This contact closes the circuit of the cut-off solenoid, the iron core, *12*, attached by a piston, *13* (to be described) to the cut-off, is drawn down against the pull of the spring, *10*, and the cut-off opens, permitting the blood collected under the emptying-plate to pass to the heart. When the vein collapses, the emptying-plate follows and the cut-off circuit is broken. The cut-off closes the vein until the cut-off contact is again made by the refilling of the vein. In this way the vein automatically fills and empties at a rate dependent on the volume-flow of blood.

This describes the operation of the instrument in its simplest form in which the cut-off solenoid alone is used. Though records of volume-flow of blood are obtainable with an instrument of this kind, a few modifications were necessary to perfect the method and to make it yield quantitative data.

In the instrument without an emptying solenoid, the vein is filled against a positive pressure (weighted emptying-plate) which assists in emptying the vein during the period in which the cut-off is open. The blood however has considerable viscosity and inertia, and therefore a constant weight which can be employed on this emptying-plate is insufficient to empty the vein completely during the short period that the cut-off is open. In addition respiratory variations of venous pressure continually obtaining on the cardiac side of the instrument vary the degree of emptying of the vein, resulting in a very irregular record. This irregularity may, however, be overcome by applying to the emptying-plate a force of sufficient strength to make the venous pressure oscillations negligible. But even so the resistance of the blood is sufficient to permit different degrees of emptying of the vein if the short period during which the cut-off is open varies only a fraction of a second. It was therefore necessary to prolong the open period of the cut-off as well as to apply a strong emptying force to the vein during this period. By prolonging the open period of the cut-off beyond that necessary to obtain complete emptying of the vein, it is unnecessary to have these periods of constant duration.

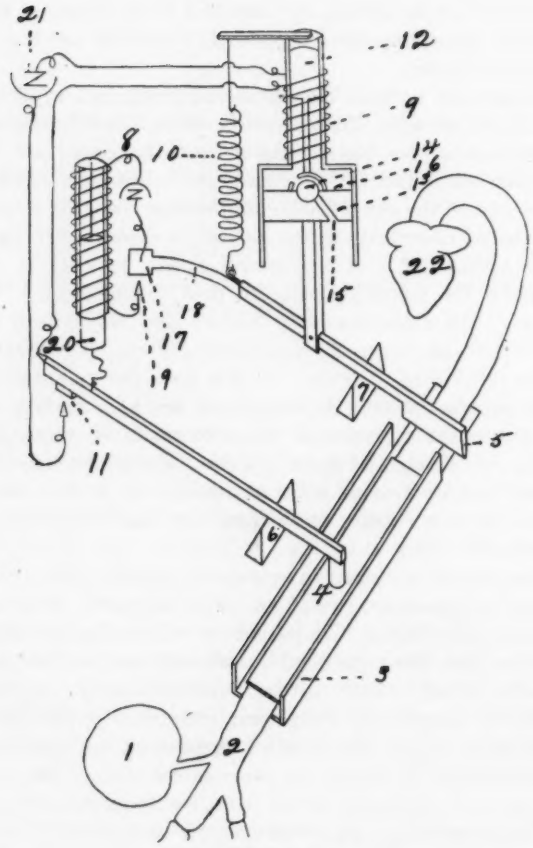

Fig. 1

Figs. 1 and 2. Schema and photograph of the automatic and bloodless volume-flow recorder. *1*, submaxillary gland; *2*, external jugular vein; *3*, trough; *4*, emptying-plate; *5*, cut-off; *6*, pivoting point of the arm of the emptying-plate; *7*, pivoting point of the cut-off arm; *8*, emptying solenoid; *9*, cut-off solenoid; *10*, closing spring of cut-off; *11*, cut-off contact; *12*, core of cut-off solenoid; *13*, cut-off piston; *14*, ball-bearing valve; *15*, opening communicating between exterior and piston; *16*, air space of cylinder; *17*, platinum plate; *18*, spring-brass strip; *19*, emptying-solenoid contact; *20*, core of emptying solenoid; *21*, batteries; *22*, heart; *23*, relay.

This period is prolonged by a piston with a ball valve, *14*, attached to the cut-off core and to the cut-off arm. The opening, *15*, connects the exterior and the air space, *16*, of the cylinder and casing of the core. The ball is freely movable and rests over the communicating opening. This arrangement permits the free entrance of air into the cylinder and a quick downward movement of the piston is possible, but at the end of the stroke the ball again closes the opening and the piston can now rise only if the air above escapes. The escape takes place through an escape valve not shown in the diagram. By the regulation of this

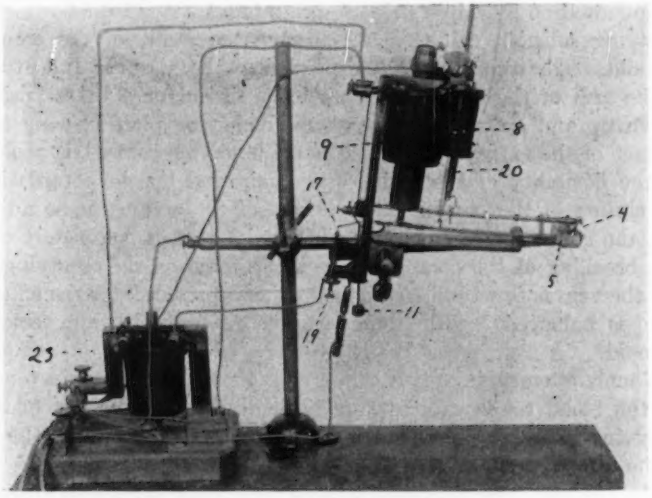

Fig. 2

valve together with the regulation of the closing spring the rate of closure of the cut-off is controlled. During the entire open period of the cut-off, a force is applied to the emptying plate by means of solenoid *8*, which brings about complete emptying.

From the previous description it is apparent that the prolongation of the open period is dependent on the length of the down stroke of the cut-off core. Though a solenoid may exert a powerful pull when a continuous current flows, this pull may be very small when only a temporary closure occurs. The duration of the closure of the cut-off contact is so short that it is necessary to prolong in a mechanical way

the period of flow of current through the cut-off solenoid. This is accomplished by placing a specially constructed relay in the cut-off circuit. The solenoid of this relay is powerful, the core is heavy, and by mounting one of the platinum contacts on a flexible strip of spring-brass attached to the core, the bending of the strip against the second contact on each closure secures the necessary prolongation.

Aside from these modifications the instrument works on the same general principles as the simpler instrument. With the vein empty and the cut-off closed, the emptying plate rises until the cut-off contact is made. But now, when the cut-off opens, a platinum plate, *17*, mounted on a flexible strip of spring brass, *18*, is bent down on the emptying solenoid contact, *19*, closing the circuit of the emptying solenoid. The iron core, *20*, suspended in the solenoid and attached to the arm of the emptying plate by a thread or a brass spring, is drawn up and exerts its force on the emptying plate so long as the contact of the emptying solenoid is made. This contact is prolonged by the flexibility of the spring-brass strip and by the retardation of the closing of the cut-off. The height of the contact is so adjusted that the contact is broken only when the cut-off is completely closed. The breaking of the contact releases the force on the emptying plate and the vein is free to fill again against zero pressure, for the emptying plate is balanced by the long arm connected with the emptying solenoid.

When the emptying-plate compresses the vein, there is a tendency for the blood to be expressed in both directions. This is overcome by giving the plate a very slight tilt which sends the bulk of the blood in the direction of the heart.

The volume-flow is graphically recorded by a signal magnet in circuit with one of the solenoid circuits. Figure 3 is a record of the volume-flow of blood of the submaxillary gland showing the effect of stimulation of the chorda tympani. The number of strokes per unit of time may be regulated to meet the demands of the experiment and the volume-flow of blood obtaining. With a rapid flow of blood a coarse adjustment is usually best while with a very slow flow a fine adjustment of the cut-off contact shows the changes in volume-flow more quickly. These adjustments are easily made by the screw showing in the photograph (fig. 2). Raising this contact diminishes the degree to which the vein fills in each interval. For qualitative work this adjustment may be freely used but for quantitative work it is necessary to calibrate the vein for every adjustment; and in doing

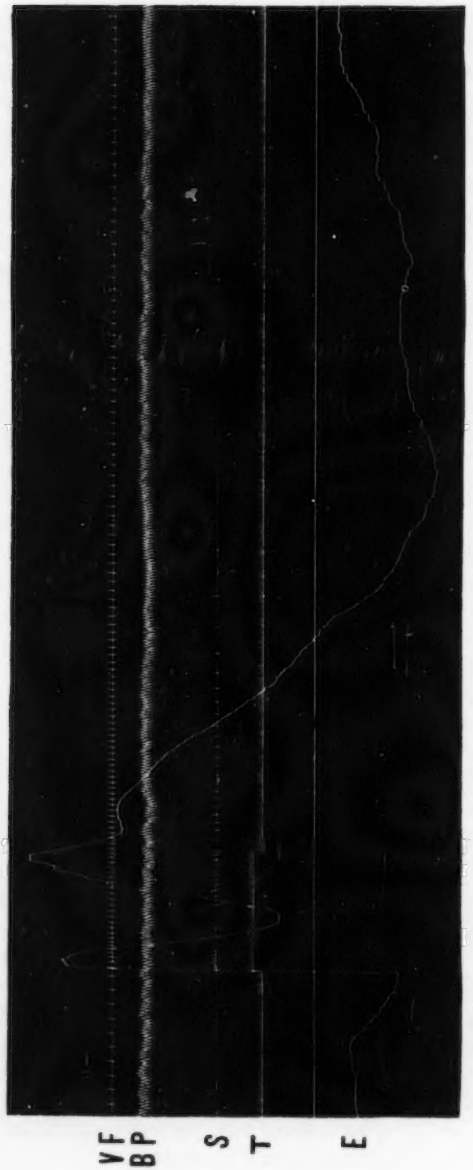

Fig. 3. Record showing volume-flow of blood from the submaxillary gland. *V.F.*, volume-flow of blood; *B.P.*, blood pressure; *S.*, salivary secretion in drops; *T.*, time in seconds, *E.*, electrical deflection.

quantitative work it is therefore desirable to set the adjustment at some mean position suitable for all conditions of the experiment.

The principle on which the instrument is constructed should permit quantitative measurements if the emptying solenoid contact is properly adjusted, and this is an easy matter. In this case all the blood passing through the vein is measured, for the vein is never completely open. It is always closed either at the lower end of the trough by the cut-off or at the upper end by the upper edge of the emptying-plate.

That the number of strokes in the record of volume-flow varies in direct proportion to the volume-flow of blood was demonstrated in

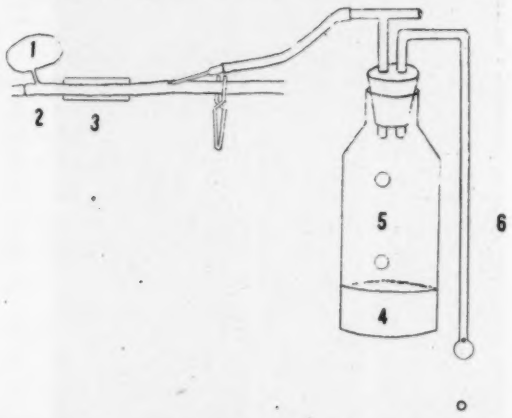

Fig. 4. Oil drop recorder. *1*, Submaxillary gland; *2*, external jugular vein; *3*, volume-flow recorder; *4*, blood; *5*, oil; *6*, siphon; *7*, dropper.

two ways: (1) The external jugular vein was isolated and connected with a burette containing saline solution. Records were then made of volume-flows of known quantities of saline solution. The number of strokes of the instrument was directly proportional, within an error of about 2 per cent, whether the flow was rapid or slow. (2) The other method consisted in measuring the volume-flow of blood through the vein in situ by the bloodless method, and in measuring it again by collecting the blood after passing the instrument. For this purpose the blood is permitted to flow from the vein through a syringe needle and rubber tube, and the drops are recorded (see fig. 4). By varying the volume-flow of blood in various ways, it was found that the results obtained by the two methods agreed perfectly.

In employing the drop method for quantitative work, difficulties in maintaining a constant size of the drop due to clotting of the blood occur. These are overcome by leading the blood into a bottle containing oil, communicating to the exterior by an overhanging siphon (see fig. 4). The blood as it enters the bottle sinks and displaces the oil. This oil is siphoned off and exerts a small suction on the vein. At the end of the siphon is a specially constructed dropper to insure a uniform drop of oil. The oil emerges at the side of the dropper as shown, instead of the bottom, and runs down the outer surface of a perfect brass sphere. Any slight change in position of the dropper will therefore have no effect on the size of the drop. An additional precaution is necessary and that is to keep the lower surface of the dropper free from clinging air bubbles. The needle which is used for drawing the blood is inserted with the bevel facing downward and is held in that position with slight upward tension on the vein, by the rod of block tin.

The two methods described for testing the accuracy of the instrument are the methods also used in calibrating the instrument in quantitative experiments.

If the first method is used (calibration by injection), the flow of blood from the tissue under study is temporarily shut off and a known amount of solution run into the vein above the instrument, the solution after passing the instrument entering the general circulation.

In the second method (calibration by bleeding), the blood passing the instrument during the period of calibration is lost to the animal.

Calibration by bleeding is the simplest method and the one to be preferred, but the method selected will depend upon the amount of blood flowing from the tissue and the degree to which hemorrhage affects the experiment. The period of calibration, at the most, need not be much over a half-minute, and in such a case as the submaxillary gland the loss of blood is negligible and therefore calibration by bleeding is the method of choice (should the loss of even small quantities of blood be detrimental to the experiment, reinjection of the blood which passes the instrument should be an easy matter). Where the blood flow is freer, as in the case of the kidney, calibration by injection is the method of choice.

If a calibration is to be of service for any length of time we must be sure that the caliber of the vein does not vary. The greatest source of caliber change is drying of the vein. This can be avoided by the use of absorbent cotton moistened in saline which, pulled out into a

thin sheet is light enough to offer no impediment to the flow of blood when placed on the vein. The whole of the exposed vein with the exception of the part in the trough is covered and with an occasional moistening of the cotton and an occasional drop of saline in the trough, the vein keeps in perfect condition through prolonged experiments up to death. Taking such precautions I have found the calibration to remain constant for periods of an hour and a half or more.

The automatic volume-flow recorder was developed primarily for studying the volume-flow of blood through the submaxillary gland. It has, however, a wider application. The blood of any of the tissues such as skin, fat, bone or muscle that can be led into a larger vein, can be measured. The instrument is so shaped that it can be inserted into the abdomen. It is therefore possible to measure with it the flow of blood through the adrenal glands, for example. In a few experiments in which the volume-flow of the kidney was measured, the method proved satisfactory. For recording the flow of this organ, the inferior vena cava serves as the reservoir under the emptying-plate. By ligating the cava at the iliac bifurcation, a reservoir of maximum size is obtained. Whether this reservoir is of sufficient size to accommodate the volume-flow of any kidney under any condition has not been determined. The application of the method to various organs and tissues is a problem in itself which has not been thoroughly looked into.

SUMMARY

An automatic and bloodless method of recording the volume-flow of blood is described.

With this method the blood, without coming in contact with any foreign substance, is measured as it flows through a vein on its way to the heart.

The blood to be measured is led into a large vein. This vein, serving as a reservoir, is placed in the trough of the volume-flow recorder.

By means of solenoids and electrical contacts operating a cut-off and an emptying-plate, the vein automatically fills and empties, the blood flowing on to the heart.

The frequency of filling and emptying varies in direct proportion to the volume-flow of blood and is recorded by a signal magnet in circuit with one of the solenoids.

The capacity of the vein lying in the trough may be varied mechanically by raising or lowering the cut-off contact. This adjustment

permits the adaptation of the instrument to different rates of blood-flow.

The method may be used to measure the volume-flow of blood from a number of tissues. Its full application, however, has not yet been worked out.

The advantages of the method are: *a*, by calibration of the vein the procedure is made quantitative without the usual direct measurements of the blood; *b*, it is automatic and therefore requires very little attention; *c*, it is bloodless and therefore does not require the use of anti-coagulants; and *d*, it may be used over long periods of time without affecting the general condition of the animal.

STUDIES ON THE SUBMAXILLARY GLAND

III. Some Factors Controlling the Volume-Flow of Blood[1]

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INTRODUCTORY

The original purpose of this paper was to study in detail certain phases of the electrical deflections of the salivary gland in an attempt to reach an interpretation of the components described in the first paper. For an understanding of the significance of these components an insight into the nature and sequence of the metabolic processes occurring during secretion, recovery and rest is needed and for that reason the metabolism of the gland was studied under varying conditions.

While this work was in progress it assumed a practical as well as a purely theoretical interest in connection with the problem of shock. Though shock may be initiated by a variety of forms of tissue abuse—anaesthesia, cold, fatigue, thirst, etc., along with inflicted injury—the disturbances set up by these forms of tissue abuse might in many instances be of lesser importance were it not for their associated circulatory disturbances. Even after largely removing the initiating causes of shock their detrimental effects on volume-flow may persist and help to sustain the condition[2].

Since normal as well as abused tissues are dependent upon an adequate volume-flow of blood the effects of decreased flow appeared to be most important, the most amenable to study and to treatment. It is a matter of some importance, therefore, to determine the flow which is adequate for normal nutrition.

[1] Reported in part before the American Physiological Society, Proceedings in this Journal, 1918, xlv, 545.

[2] See page 469 of paper IV for a fuller discussion.

Fig. 1. Record showing parallel fluctuations in secretion, volume-flow of blood and electrical-deflection elicited by prolonged chorda stimulation. *V.F.*, volume-flow of blood; *B.P.*, blood pressure; *S.*, secretion; *T.*, time and stimulation, *E.*, electrical deflections.

The question of determining the minimal flow of blood necessary to maintain the tissues in a normal condition is by no means an easy one for we know that tissues, even though the flow of blood is very low or even absent, may vigorously respond to stimulation. Tissues therefore, evidently possess a factor of safety both in the form of stored energy and in the form of ability to work under unfavorable conditions for a long period of time without sustaining injury, but on the other hand there is the possibility that tissues apparently responding in a normal way may be undergoing a progressive decline. It seemed that this question could be elucidated in part by a study of the mechanism regulating the volume-flow in both active and resting tissue by the methods developed in the study of electrical deflections of the submaxillary gland.

The view that vaso-dilator nerves are of fundamental importance in producing increased blood-flow during tissue activation is accepted by many. Others believe that the action of vasodilator fibers has not been definitely proved and that the increased metabolism always precedes and is the peripheral chemical cause of the increased flow of blood. (Gaskell, Barcroft and Henderson and Loewi and others.)

It was this problem of the mechanism of the control of volume-flow of blood, begun in connection with the observations of the synchronous fluctuation of the electrical deflection, of secretion and of volume-flow of blood accompanying prolonged chorda stimulation that I was studying when the interest in shock developed. (See fig. 1.) It seemed that the two problems, the theoretical and the practical, might be followed simultaneously and it should be mentioned here that the results in this paper will be considered primarily from a theoretical point of view. Their practical application will be considered in the following paper.

THEORETICAL

When the chorda lingual nerve is stimulated for a period of a minute or more and the associated electrical deflection recorded, crests 1, 2 and 3 usually appear. If the secretion and the volume-flow of blood are likewise recorded fluctuations corresponding with crests 2 and 3 occur. (See fig. 1.)

These fluctuations in secretion may be variously explained. They may be due to causes other than the synchronous changes in volume-flow of blood but if volume-flow is a factor it is a question of theoretical and practical interest to know the sequence of events; whether

increased volume-flow of blood is primary, accelerating secretion, or whether it is secondary to an accumulation of metabolites accelerating the volume-flow of blood.

Causes of secretory fluctuations during prolonged chorda stimulation. *a.* The fluctuations in secretion may be the result of inhibitory action of the chorda tympani fibers upon the secreting cells; thus Langley (1) found after incomplete paralytic doses of nicotine that salivary secretion produced by chorda stimulation was mainly an after-effect. Dale and Laidlaw (2) likewise noted that if the chorda tympani is stimulated during the rapid secretion occurring at the cessation of the

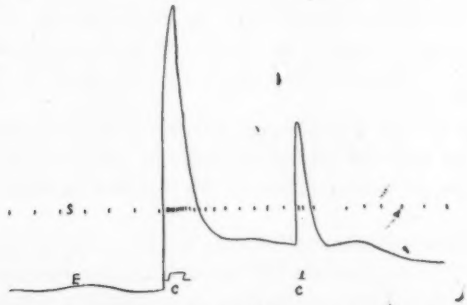

Fig. 2. After-secretion showing effect of short chorda stimulation. *S.*, salivary secretion, *E.*, electrical deflection, *C.*, chorda stimulation.

original stimulation, the secretion is again retarded. Applying this observation to more normal conditions, they state that

> there is an indication of double effect of the chorda even before the administration of an alkaloid, in that the rate of secretion falls off towards the end of prolonged stimulation to be accelerated again at the end of stimulation.

In this research these results have been repeatedly confirmed. See the effect of prolonged chorda stimulation on secretory fluctuations in figure 1 followed by an after-secretion. In figure 2 note the effects of short stimulation during the period of after-secretion. The primary increase in rate of salivary flow suggests a contraction of the ducts expressing the contained secretion. The subsequent period of retarded flow suggests a dilatation of the ducts taking up the steady after-secretion. These results bring into question whether in the case of prolonged chorda stimulation the slowing of secretion following the first

rapid secretion is an effect of secretory inhibitory fibers or is due to changes in caliber of the salivary ducts.

b. The fluctuations of secretion may also be connected with variation in the amount of water directly at the disposal of the cells for secretion; for Barcroft (3) found it necessary to ignore the first minute of stimulation to obtain a constant relation between the amount of saliva secreted and the amount of water lost by the blood in passing through the gland. The observations of Bunch (4) on volume changes in the gland during chorda stimulation point in the same direction.

c. A possible factor operating to produce secretory fluctuations is the effect which a sudden increase in metabolism itself has upon subsequent metabolism, as demonstrated in the staircase phenomenon in a series of muscular contractions. A concentration of metabolites developed during the period in which the pre-formed saliva is being liberated might have an effect upon further response to prolonged chorda stimulation.

d and *e.* Two other possible explanations of the fluctuation in secretion have to do with the interdependence of volume-flow of blood and metabolism, the particular phase of the problem in which we are now interested.

The second increase in secretion may be due to the second increase in volume-flow of blood, but on the other hand the increase in volume-flow of blood may follow an increase in metabolites resulting from the increased secretion.

To throw light on this question of the control of volume-flow and on the dependence of nutrition on volume-flow certain mechanical, nervous and chemical factors affecting flow of blood were studied.

The effect of arterial compression on the response of the gland to stimulation of the chorda tympani. Interference with the blood-flow to the gland should permit the study of combined effects of ischaemia and of greater concentration of metabolites during chorda stimulation. Though considerable work has been done on this problem (5), (6), (7) and (8), I was interested, during the study of electrical deflections in another phase, namely, the effects of arterial compression on the electrical deflections. Some results then obtained are given here.

Assuming that compression of the artery invariably decreases the volume-flow of blood, we might expect a uniformity of results. For the most part a decrease in the response of the gland to stimulation occurred but striking exceptions were noted. I hesitate to describe these results for the reason that they have not been noted by others

and that later when an interpretation of the results was sought by a synchronous record of the volume-flow of blood, they could not be duplicated. They must, therefore, be taken for what they are worth.

Figure 3, *A*, of experiment 46 shows the results of chorda stimulation with and without compression of the artery. Compression in each instance accelerates secretion with an apparent alteration of the processes of secretion. At least this is suggested by the electrical deflec-

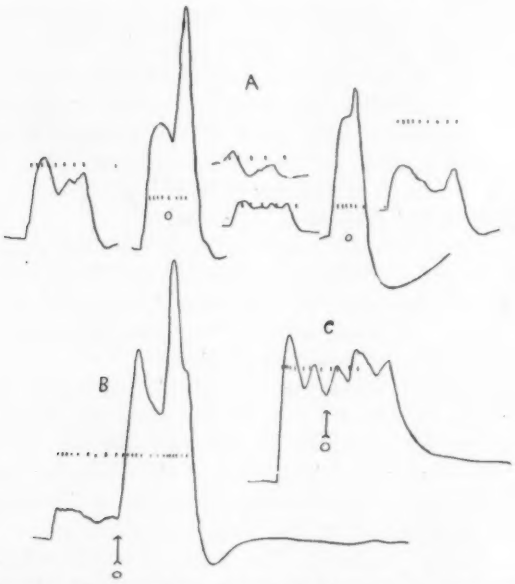

Fig. 3. Records showing effect of arterial obstruction on secretion and electrical deflection with chorda stimulation. The records marked *O* were obtained during obstruction. Figures 3 *A* and *B* are from exp. 46, figure 3 *C* from exp. 47.

tion, for the deflection on compression is increased out of all proportion to the acceleration of the secretion.

Figure 3, *B*, of experiment 46 shows the effect of carotid compression during prolonged chorda stimulation. The acceleration in secretion and the disproportionate increase in the deflection occur again. Another point in this record exciting speculation is that although crests 2 and 3 appear during chorda stimulation before obstruction, they are duplicated by arterial obstruction. In figure 3, *C*, experiment

47, the enhancing effects of arterial compression are small and transitory yet the acceleration of secretion with the duplication of crests 2 and 3 occurs again.

The experiments indicate the possibility of a transitory stimulation by certain grades of ischaemia. The possibility that the response might be due to an effect of central asphyxia transmitted to the gland through the sympathetic fibers occurred to me too late to make the necessary controls.

Observations on the volume-flow of blood during prolonged chorda stimulation without carotid obstruction. While some of the results of arterial compression might suggest that certain grades of asphyxia have an enhancing effect on secretion, the records of volume-flow during chorda stimulation in agreement with most of the results obtained with arterial compression, seem to suggest the reverse. For they show fluctuations in volume-flow running parallel with the fluctuations in secretion and electrical deflections. (See fig. 1).

The interpretation of these results depends upon the exact sequence of changes in secretion and in the volume-flow of blood and upon the extent the two are dependent on each other. An answer to this question might decide the issue between the metabolite and the vasomotor nerve control of volume-flow of blood.

The fluctuations in volume-flow are probably not due to the abstraction of water from the blood, for in the first place the secretion formed is not as great as the fluctuation in the volume-flow. In the second place, identical changes in volume-flow of blood can be elicited by a weak stimulation that fails to produce secretion in the normal gland, and by strong stimulation of the chorda tympani after a paralytic injection of atropin sulphate. The fluctuations in volume-flow of blood, therefore, appear to be vasomotor phenomena, either of nervous or metabolic origin.

a. It is difficult to devise a direct experiment to determine the time relations between vasodilatation and metabolism (secretion) owing to the difficulty in determining the moment of vasodilatation. The latent period of secretion elicited by chorda stimulation as measured by the appearance of saliva is variable but when measured by the electrical deflection the response of the gland is prompt, so that the moment of stimulation marks closely the beginning of secretion. The latent period of dilatation as measured by increased volume-flow of blood is prolonged by the filling of the capillary bed.

b. Atropinization experiments seemed for a time to give direct evidence of independent systems of fibers, vasodilator and secretory, for after apparently paralyzing the secretory function of the chorda tympani fibers, electrical stimulation still produces an increase in volume-flow of blood suggesting the unimportance of metabolites. Barcroft (9), however, finds even after atropinization chorda stimulation still accelerates the gaseous metabolism of the gland and, therefore, the metabolite theory of vasodilatation is not invalidated.

c. Another apparently direct method which has been used to demonstrate the presence of two sets of fibers is the grading of stimulation. Fibers differing so much in function as secretory and vasodilator fibers might well differ in their threshold of stimulation. A number of workers have elicited, with weak stimulation, an increased volume-flow of blood without an accompanying secretion, concluding the presence of both types of fibers in the chorda tympani. I have had no difficulty in duplicating these results, but synchronous records of the electrical deflections suggest that even though the conclusion regarding the presence of the two sets of fibers may be correct, it is not well founded. When a stimulus just below the threshold for visible secretion is employed, a typical deflection, though not as high as the deflection accompanying secretion, is obtained. Though with decreasing strength of stimulation the deflection decreases in amplitude along with the decrease in volume-flow of blood, it still possesses its usual contour. The deflection and the increased volume-flow of blood finally disappear together, therefore, if the two sets of fibers do exist they appear to have the same threshold of stimulation; or, stated in another way, with chorda stimulation we never obtained increased volume-flow of blood without an accompanying increased metabolism.

The validity of this statement depends upon the interpretation of the electrical deflection and it should be stated here that the deflections cannot be accounted for by changes in volume-flow of blood per se.

The direct methods of measuring the latent periods of secretion and vasodilatation, of paralyzing one set of fibers, leaving the other intact, and selective stimulation by grading of stimulation, have failed, therefore, to give conclusive data. A further search for direct methods proving unsuccessful, an endeavor was made to gain the desired information by indirect methods. It was hoped that in this way a mass of indirect evidence might be obtained from which conclusions in favor of

one or the other of the two theories of volume-flow control might be reached.

The relation of volume-flow of blood to superbasal metabolism with a constant head of blood pressure. Chorda stimulation produces vasodilatation by inhibition of the intrinsic and central tonus of the vessels. Assuming the tonus to remain constant the volume-flow of blood might roughly vary as the intensity of chorda stimulation irrespective of whether the control is chemical or through vasomotor nerves.

However the results from vago-sympathetic stimulation, as is well known, vary. In the dog secretion is almost invariably accompanied by a decrease in volume-flow of blood, while in the cat it often is associated with an increased flow. Barcroft (10) pointed out that the difference in the results depends upon the balance of the vasoconstrictor effects of the constrictor fibers and the dilator effect of the metabolites liberated in secretion. Since the sympathetic secretion is very much more copious in the cat, the metabolites gain the upper hand. Carlson (11) takes exception to this view. Employing methods of selective stimulation he concludes that the cervical sympathetic of the cat contains vasodilator fibers as well as vasoconstrictor and secretory fibers.

The same question with regard to the relation of volume-flow control to vago-sympathetic stimulation arises in the case of the dog for in a few experiments I have observed that stimulation of the vago-sympathetic, strong enough to produce secretion, has elicited an increased after-flow of blood. (See fig. 4).

Langley (12) found that combined stimulation of the chorda tympani and the vago-sympathetic may elicit secretion equal to the sum of separate stimulations. Von Frey (13) found that the volume-flow of blood with combined stimulation was less than the flow elicited by chorda stimulation though it may be greater than the flow of rest.

By recording simultaneously the volume-flow of blood and the secretion with separate and combined stimulation of the chorda tympani and the vago-sympathetic, I have found that the results of Langley and Von Frey may be combined; that is, that with combined stimulation of the two sets of nerves we may get a summation of secretion with a flow of blood less than that elicited by chorda stimulation alone. (See fig. 4).

It is apparent, then, that the quantitative relation between increased metabolism and increased volume-flow of blood is a variable one depending upon the conditions of the experiment; that the vasoconstric-

Fig. 4. Three records showing separate and combined effect of chorda and vago-sympathetic stimulation on the secretion, volume-flow of blood and electrical deflections. An increased after-flow of blood following vago-sympathetic stimulation is also shown.

tor tonus and its inhibition is an important factor in controlling the volume-flow. In making a quantitative study of the relation of metabolism to volume-flow of blood, it is best to activate the gland by stimulating the chorda tympani nerve in which there are no or at least few constrictor fibers. Under such circumstances we can assume the potential vasoconstrictor tonus to be constant.

Assuming that metabolites control the volume-flow of blood, it might be fair to expect the flow to bear a direct relation to the amount of metabolism. With chorda stimulation such a relation seems to be more essential for the metabolite mechanism of control than for the nervous and therefore, if found, should add support to the former theory.

In the method used to study this relation it is presumed that the resting or basal metabolism of the gland calls for just enough blood to carry on that metabolism in a normal fashion. Any extra flow of blood produced by activating the gland is called for by the extra or superbasal metabolism. With the general condition of the animal constant, the chorda tympani was stimulated at regular intervals of ten minutes, and one, two, four, six, etc., drops of saliva elicited by varying, but relatively short, periods of stimulation. The salivary secretion was recorded by the drop method and the size of the drop clinging to the dropper at the end of secretion estimated. The volume-flow of blood was recorded with the bloodless method to insure a constant condition of the animal. Subtracting the basal flow of blood for a period of five minutes preceding stimulation from the flow—basal plus superbasal—of the following five minutes initiated by the stimulation, we get the secretory or superbasal flow. Plotting the superbasal flow on the ordinates against the amount of secretion on the abscissas we find the relation of flow to metabolism to be represented by a straight line. (See fig. 5).

When the secretory blood-flow is divided by the amount of secretion we do not get a constant. This interesting feature is shown in the curve. It will be noted that the curve at zero secretion does not end at the intersection of the ordinates and abscissas but rather at a point on the ordinates above the abscissas, in agreement with the observation cited before that even though chorda stimulation may be too weak to elicit secretion yet the electrical deflection accompanying the increased flow of blood indicates that the gland has been activated.

Though not necessarily ruling out the nervous regulation of blood vessels, the linear relation of volume-flow of blood to glandular metab-

olism is suggestive of a purposive mechanism of volume-flow control. The value of the results in their bearing on the theory of control is to some extent a matter of opinion—the question is whether or not we might expect so fine an adjustment of flow with stimulation of vasomotor fibers entirely independent of metabolism. Would one be justified in expecting, under independent control, an occasional disturbance of adjustment? For this reason the observations were

ERRATA

[To be inserted between pp. 448 and 449, Vol. xliv.]

Page 449, line 20, for "$\frac{\theta_m \quad 1}{0.825 \ . \ \text{wt.}}$" read "$\theta_m \times$ sp. ht."
line 23, the equation should read, $1.3 \times 10^{-3} \times .83 = 11. \times 10^{-4}$ cal.
line 26, the equation should read, $9.2 \times 10^{-4} \times .83 = 7.6 \times 10^{-4}$ cal.
Page 450, the upper equation should read, $1.2 \times 10^{-3} \times .83 = 9.9 \times 10^{-4}$ cal.
In the second equation for "4.8" read "11.2," and for "1.3" read "3.0."
In the second line above the italicized paragraph for "1.55" read "3.6," and for "4.2" read "9.8;" in the paragraph itself for "0.155" read "0.36," for "0.042" read ".098."
In the seventh line from the bottom for "a gratifying agreement with" read, "a number nearly one-half of".
Page 451, line 1, for "ten", read "four".
line 9, delete entire paragraph.
Page 452, line 8, for "0.00048" read "00.10."
line 10, for "0.155" read ".36."
line 12, for "4.2" read "9.8."

The relation of basal flow of blood to basal metabolism. To test the assumption made above, that the basal flow of blood also bears a linear relation to basal metabolism the effect of depriving the gland of its normal flow of blood was studied. I hoped by producing asphyxial conditions of different degrees (time of obstruction) and measuring the after acceleration of blood-flow during recovery when the obstruction is removed, that a quantitative study of the relation of volume-flow to basal metabolism might be made.

The effects of partially obstructing the artery for a few minutes are shown in figure 6 in which the volume-flow of blood is plotted on the ordinates against time on the abscissas. After diminution of the

volume-flow to the minimum at *B* there is a steady acceleration up to *C*, where the obstruction is removed. Then follows a flow considerably faster but soon returning to normal.

The effects of obstruction are striking and the method, therefore, gave promise of yielding quantitative data. But a difficulty in interpretation became apparent, in the form of an additional variable,—the effect of tension on the tone of blood vessels—making a quantitative study of the effect of asphyxia difficult. The effect of tension on blood vessels was pointed out by Bayliss (14). The same effect of tension on the tone of the auricle of the turtle was also noted by myself (15). Increasing the filling tension of an automatically beating auricle suddenly increases the auricular volume, followed by a development of tonus as shown by a slowly decreasing volume. These volume curves resemble somewhat the curve of volume-flow of blood in figure 6. This method for determining the relation of basal-flow to basal metabolism, therefore, is not quantitative, for the question naturally arises, how much is the acceleration of blood flow from *B* to *C* and at *D* due to the diminution of tone from concentration of metabolites or to decreased tension; and how much of the decreased flow from *D* to *E* is due to a washing out of metabolites or to increased tension.

The relation of superbasal flow of blood to superbasal metabolism with decreasing blood pressure. The observations on this point have a double interest; one, in the relation to the mechanism of volume-flow control, the other in the relation to the actual amount of superbasal-flow for a given amount of work at progressively lowered blood pressures. Here we are interested mainly in the former question.

With all other conditions of the animal kept as constant as possible, equal amounts of secretion were elicited by chorda stimulation at progressively lowered blood pressures secured by haemorrhage. It was hoped that by plotting the curves of secretion and the curves of volume-flow the mechanism of volume-flow might be suggested. If vasodilatation and metabolism vary independently of each other, the amplitude of the volume flow curve on account of the diminution in the driving pressure, would be diminished but the time relations of the two curves would remain the same. If metabolites have an effect, the amplitude of the volume-flow curve may likewise be reduced, the accelerated volume-flow of blood, however, would be prolonged until the metabolites were disposed of, counteracting the decreasing blood pressure and tending to keep the superbasal-flow constant.

A series of curves of secretion and volume-flow of blood are shown in figure 7. In each instance approximately eight drops of saliva were elicited. The blood pressures at which stimulation occurred are designated. The curves beginning above the base line are curves of volume-flow of blood, the horizontal portion preceding chorda stimulation representing the basal-flow of blood. The triangular portion above the basal-flow represents the superbasal-flow (for data see table 1). With decreasing blood pressure the area of the triangle diminishes and at about 34 mm. Hg. the flow of blood is no longer increased by chorda stimulation. At lower pressures the flow is actually decreased.

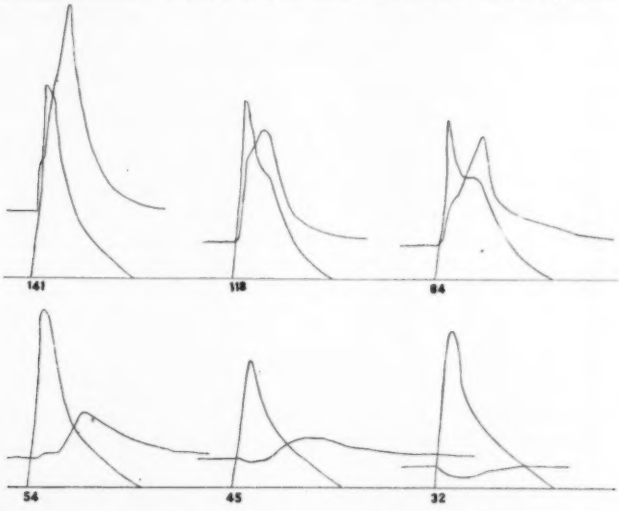

Fig. 7. Curves showing both basal and superbasal-flow of blood at varying designated blood pressures.

The practical importance of these results in conditions of lowered blood pressure is obvious. In relation to the theories of volume-flow control the fact that the superbasal-flow decreases so rapidly and that the effect of decreased blood pressure is not better counteracted seems to speak against metabolite control—but the very great importance of pressure not only as a driving force, but also as a stretching force, the increased construction of central origin from haemorrhage and the possibility of passive constriction with decreasing head of pressure, might overrule this objection and will be discussed later. The

fact that the decreased pressure is somewhat counteracted, as evidenced by the prolongation of the after-flow, indicates that the volume-flow of blood is in part, at least, dependent on metabolites.

The relation of basal-flow of blood to mean blood pressure. There seems to be little quantitative data on record on the relation of basal-flow of blood to decreased blood pressure produced by haemorrhage. If driving pressure is the only variable, the volume-flow of blood would steadily decrease and when plotted on the ordinates against blood pressure on the abscissas would be represented by a straight line. If a deflection of this curve occurs some other variable has come into play.

TABLE 1

OBSERVATION	BLOOD PRESSURE IN MM. Hg	BASAL BLOOD FLOW IN DROPS PER MINUTE	SECRETORY BLOOD FLOW + SECRETION DIVIDED BY SECRETION
6	141	39.5	9.5
11	129	27.7	8.6
14	115	21.0	5.8
16	84	20.0	5.8
20	60	19.0	6.8
21	54	18.0	3.7
22	49	17.2	3.7
23	45	18.0	3.3
24	41	17.4	2.1
25	34	17.2	1.0
26	34	16.6	0.0
28	32	16.0	Lower than basal flow
29	29	13.7	
30	28	11.1	

These experiments were performed to find how the volume-flow of blood is affected by a fall in pressure, in other words whether the dilating effect of metabolites might be demonstrated by a bending of the curve, and by this bending the volume-flow of blood necessary for normal tissue metabolism might be determined. It seemed that the volume-flow might decrease with the fall in pressure up to a point where the flow of blood was no longer sufficient to carry off the metabolites, permitting a sufficient concentration to affect vasodilatation, or an upward bend of the curve. This would counteract the decreasing head of pressure and tend to keep a constant volume-flow acting as a protective mechanism to maintain adequate nutrition. The point at which the rapid decrease in volume-flow of blood is checked

by the hypothetical concentration of metabolites would indicate approximately the volume-flow essential to the tissues.

The basal-flow of blood was measured by the drop method and plotted on the ordinates against mean blood pressure on the abscissas. (See fig. 8.)

Since the flow of blood is not represented by a straight line, according to the law of Poiseuille, driving pressure cannot be the only variable. One or more additional factors equally as important enter into play dividing the curve into three distinct parts—the first, in which the flow decreases decidedly faster than the fall in blood pressure; the second, or the plateau, in which the rapid decrease is checked; the third, in which the flow again decreases faster than the fall in pressure.

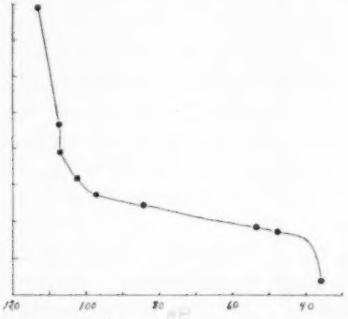

Fig. 8. Curve of basal flow of blood obtained in a haemorrhage experiment. Flow is plotted on the ordinates against blood pressure on the abscissas.

The change in flow during the second period is variable. The flow may decrease but always much more slowly than the decrease in pressure, it may remain constant or in some instances actually increase. Despite the agreement of the curve with the working hypothesis, a checking up of the factors controlling volume-flow seemed called for.

The three main factors determining the rate of flow are driving pressure, viscosity of the blood and caliber of the vessels.

Viscosity. The viscosity of the blood depends primarily upon the number of corpuscles. In haemorrhage there is a progressive dilution of blood from the outset. The rapid fall in volume-flow cannot be attributed to, but actually is counteracted by dilution of the blood. That the sharp bend in the curve cannot be attributed to the counteracting influence of dilution is also certain. In some animals in which

the blood dilutes rapidly the decreased viscosity may be a factor of some importance, but in these experiments on the dog in which the percentage of corpuscles was followed, the slow and even dilution of the blood rules out viscosity as an important factor. (See fig. 4, exp. 87, page 485.)

Injecting 7 per cent gum acacia to raise the pressure after extensive haemorrhage produces a volume-flow of blood greater for the same head of pressure during the haemorrhage. This may be explained either by a dilution of the blood decreasing the viscosity or by a dilatation of the vessels permitting a greater flow. The point of interest is that the contour of the injection curve of flow duplicates the haemorrhage curve. (See fig. 7, exp. 49, page 490.)

The converse also holds. When the blood pressure is reduced by tissue abuse it is accompanied by a concentration of the blood yet the curve of volume-flow is essentially the same as in haemorrhage. Changing viscosity of the blood, therefore, affects but little the contour of the volume-flow curve. (See fig. 7, exp. 48 and 40, page 490.)

Caliber of the vessels. The breaks in the curve, therefore, are due mainly, to a change in caliber of the vessels. In part 1 there is a constriction; in part 2 a checking of this constriction, in part 3 a second constriction.

The gradient of the first part of the curve and the point at which it is checked varies in different animals and apparently depends upon a number of factors—the tonus of the blood vessels at the beginning of haemorrhage and the relative importance of active and passive constriction. In haemorrhage active constriction is a well-known reaction. One frequently meets with animals reacting to a large haemorrhage with a rise instead of a fall in blood pressure, the volume-flow of blood markedly decreasing.

Every effort was made to avoid complicating the curve with the effects of cold and of inflammation. In the first place, the gland is dissected carefully. Frequently, when the veins are conveniently placed, the dissection requires no manipulation of the gland whatever. The gland is re-covered by the overlying skin and a protecting layer of gauze. That a more or less permanent change in the vessels, such as is associated with inflammation, does not occur is shown in the re-injection experiments, where the volume-flow curve with decreasing pressure is duplicated on increasing the pressure by gum acacia injection. (See fig. 6, page 449, and fig. 7, exp. 49, page 490.)

The decrease in the volume-flow of blood in the first part of the curve

during haemorrhage with a rise in pressure can therefore be safely attributed to active vasoconstriction of central origin. This active constriction may continue with the fall in blood pressure. The bend in the curve initiating the plateau may mark the point where the vasomotor center is losing its tonus. It may also mark the point where the concentration of metabolites becomes such that its peripheral dilating effect counteracts the constriction and fall in blood pressure.

The effect of vago-sympathetic section on the curve of volume-flow. By measuring the volume-flow of blood from both glands with the vago-sympathetic intact on one side and cut on the other we have apparently a simple method for determining the relative importance of vasomotor changes of central or peripheral origin in different parts of the volume-flow curve. But the effects of section of one nerve proved to be a problem in itself and were followed out only in part at this point. The eight experiments performed, however, were of some value in furnishing material for the study of volume-flow control and are therefore described.

The flow was measured by the drop method; the animal was bled continuously up to death through the two glands. At the beginning of the experiment the vago-sympathetic on the right side was cut.

A few experiments showed that the effects of section of the vago-sympathetic were not always limited to one side alone but that dilatation of varying degree occurred on the contra-lateral side as well. On section of the nerve, the volume-flow increases rapidly, reaching its maximum in about a minute and then gradually decreasing. The normal tone on the side of the cut nerve is usually reached in about five to seven minutes. These points are brought out in figure 9 and table 2. In experiment 79 the effect of section of the vago-sympathetic is apparently limited to the right side alone. The slight increase in volume-flow of the left side can be attributed to the increased blood-pressure. On the right side the maximum flow is 333 per cent of initial flow, but in five minutes when the initial blood pressure is reached again the flow is 54 drops per minute, or the initial rate The tonus of the vessels of the left gland in the meantime has increased beyond the normal, the flow decreasing from 36 to 26.6 drops per minute.

In experiment 78 the effect of nerve section is definitely bilateral, for the blood pressure remains constant, yet the flow on the left side is increased. In experiments 77 and 80 (no record shown) the contra-lateral dilatation is very marked.

Fig. 9. Records showing homo- and contralateral effects of vago-section on the volume-flow of blood through the submaxillary glands.

If we examine the curves of volume-flow of blood from experiments 77 and 80 we find that the contour is very similar to the contour of curves obtained with the vagi intact, and it is surprising to find the curves of flow of the two sides so nearly alike as shown in the curves from experiment 77. In experiment 80 where the initial flow of the two glands (right 66.6 and left 98.4 drops per minute) varied considerably—right vago-section increased the flow of the two glands to

TABLE 2

EXPERIMENT 79			EXPERIMENT 78			EXPERIMENT 77			EXPERIMENT 80		
B. P. in mm. Hg	Rt. V. F. in drops per min.	L. V. F. in drops per min.	B. P. in mm. Hg	Rt. V. F. in drops per min.	L. V. F. in drops per min.	B. P. in mm. Hg	Rt. V. F. in drops per min.	L. V. F. in drops per min.	B. P. in mm. Hg	Rt. V. F. in drops per min.	L. V. F. in drops per min.
108	54.0	36:0	112	29.0	27.0	116	85.0	126.0	110	66.6	98.4
Cut	Rt.	Vagus	Cut	Rt.	Vagus	Cut	Rt.	Vagus	Cut	Rt.	Vagus
121	180.0	41.2	116	90.0	33.0	117	195.0	198.0	105	128.0	130.0
110	54.0	26.6	114	63.5	28.5	121	159.0	152.0	102	110.0	110.0
104	54.0	31.0	107	40.8	24.5	116	105.0	112.0	102	90.0	90.0
100	63.0	33.0	93	25.5	16.3	110	85.0	96.0	100	78.0	79.0
97	61.0	32.0	96	26.3	19.6	105	80.0	90.0	94	56.6	56.6
93	58.4	31.0	87	34.5	23.5	100	73.0	70.0	78	48.0	45.0
92	58.0	31.0	84	36.0	23.0	95	72.0	68.0	72	44.0	43.7
82	55.6	28.6	74	30.5	21.0	90	62.0	50.0	64	38.6	38.4
76	48.5	27.7	63	27.5	22.0	84	46.0	36.0	57	32.4	34.3
71	44.0	25.0	60	22.0	19.6	78	38.0	30.0	50	26.8	28.4
64	37.0	24.0	50	18.8	18.3	70	36.0	26.0	34	18.5	21.4
60	32.5	22.0	41	19.0	18.3	62	27.0	14.0	20	15.0	16.0
55	30.3	21.4	30	38.0	14.0	50	23.0	13.0			
40	26.2	16.0				43	21.0	12.+			
24	29.0	22.0				37	21.5	13.3			
21	35.0	13.4				27	19.2	11.4			
						25	17.6	10.0			
						18	7.1	5.8			

128 and 130 respectively. On bleeding, the flow from both sides remained so nearly alike at most pressures that only one curve was plotted. (See table 2.) Apparently the function of the vago-sympathetic in this experiment is thrown out more or less completely on both sides, so that both curves may possibly be looked upon as unaffected by central increased tonus working through the vagus nerves. Though in these experiments we get no decreased volume-flow with an elevated pressure or stationary pressure, except where the vessels

are gaining lost tone, curves 77 and 80 show that a definite vasoconstriction is taking place. This constriction may be passive and due to a decreased stretching head of pressure. Owing to the conflicting data on the action of adrenin in haemorrhage the effect of this hormone cannot be used to explain the results (16). The curves show, at least provided no constriction occurs through the chorda tympani, that a fall in blood pressure when unaccompanied by an active central

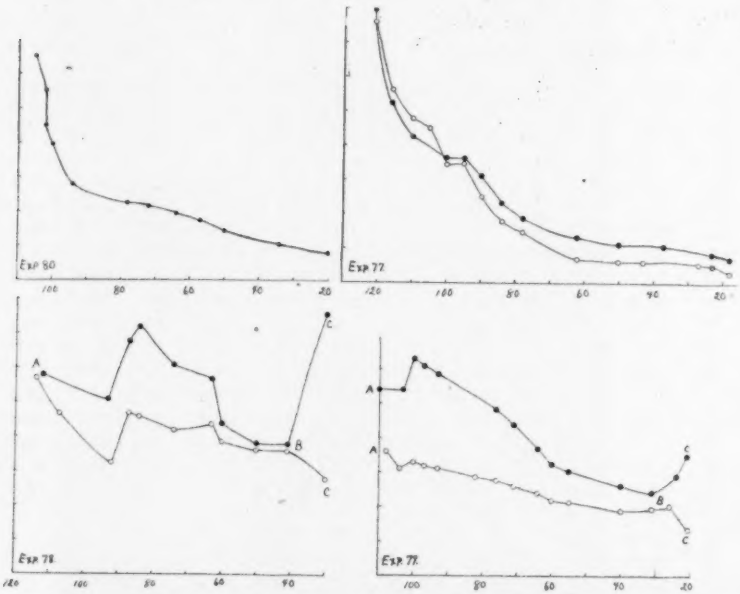

Fig. 10. Curves of basal-flow of blood from both submaxillary glands showing the effect of section of the vago-sympathetic on the right side.

constriction has serious effects on the volume-flow of blood out of all proportion to the fall in pressure.

The curves of flow of experiments 78 and 79 show similar irregularities which are also present though not as well marked in the remaining experiments on section of the vago-sympathetic. These irregularities have not been explained but the failure of the volume-flow to decrease in the usual way with decreasing head of pressure suggests some peripheral chemical effect such as might result from disturbed metabolism

following vago-section or some central effect working through the chorda tympani, an effect which was not ruled out.

In experiments 78 and 79 the effects of vago-section were mainly homolateral yet the configuration of the curves from *A* to *B* in each pair is very similar. From *B* to *C*, however, there is a marked divergence—a rapid increased volume-flow on the side with the nerve cut though the pressure is falling and a sudden diminution in flow on the side with the innervation intact, suggesting a peripheral chemical dilatation in one gland and an overcoming of this by constriction of central origin in the other.

The relation of alkaline reserve to volume-flow of blood. This divergence of the two curves brings up the question of the nature of the metabolite producing the reaction. The liberation of powerful dilating substances such as have been studied by Dale and Laidlaw (17), Dale and Richards (18) and Hunt (19) offers the possibility of an effective mechanism of dilatation during tissue activation. We must look to other substances, however, to explain this double reaction.

We know that in conditions of lowered blood pressure, particularly when the respiratory center fails to respond, an increase in hydrogen ion concentration develops as a result of the decreased carbon dioxide capacity of the blood and the increase in concentration of carbon dioxide. And we know the central constricting and peripheral dilating effects of carbon dioxide. Mathison (20) states that there appears to be no reason for regarding the respiratory center as a particular mechanism alone possessing the property of responding to small changes in CO_2 tension of the blood. Hooker, Wilson and Connet (21) found that chemicals which excite or depress the respiratory center have the same effect upon the vasomotor center. The absence of a rise of pressure from the administration of carbon dioxide after decerebration was shown by Kaja and Starling (22) and by Henderson (23). Hooker (24) demonstrated the peripheral dilating effect of CO_2 in the perfused pithed frog and also (25) found CO_2 to have a specific effect, due to other than its acid properties, on the respiratory center of the dog. These observations have more recently been confirmed by Scott (26).

The dilating effects of acid injection can be demonstrated when the vago-sympathetic is cut as is shown in figure 11. The injection presumably increases the hydrogen ion concentration as is indicated by the increased pulmonary ventilation. When the vagi are intact, however, the results obtained are variable. Sometimes the flow was

increased, unaffected or decreased. The decreased flow presumably is a result of constriction of central origin as is suggested by an accompanying rise of pressure.

Relation of volume-flow to head of pressure in perfused dead organs. The first segment of the curve of volume-flow of blood in the haemorrhage experiments may represent combined active and passive constriction, the second segment may represent a cessation of both, giving way to a dilatation of central and peripheral origin. It seemed to the point, therefore, to determine the relative importance of physiological reactions and purely mechanical factors.

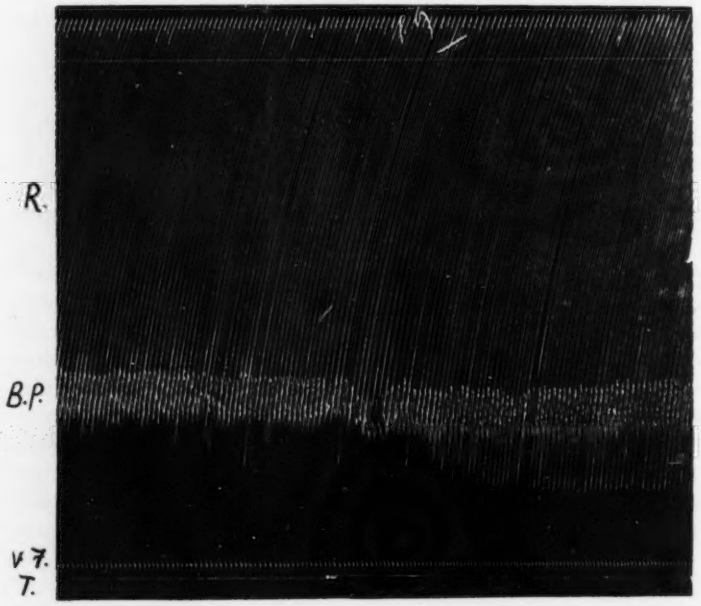

Fig. 11. Record showing the effect of acid injection on respiration and on the volume-flow of blood through the submaxillary gland with the vago-sympathetic cut.

Exceptions to Poiseuille laws have been noted by Denning and Watson (28) and others; e.g., Denning and Watson find that the flow of fluids through inextensible tubes, when plotted on the ordinates against head of pressure on the abscissas, is not represented by a straight

line but by a curve with the convexity down. The extent to which this bending occurs increases with the decrease in caliber of the tubes and with an increase in suspended solid particles in the fluid. Such exceptions along with the possibility of a passive constriction of blood vessels accompanying a fall in blood pressure suggested the advisability of artificial perfusion of dead tissue. Kidneys of the dog and cat, excised from one to three days previously, were perfused at room temperature with ox-blood diluted with 7 per cent gum acacia in 0.9 per cent NaCl and also with gum acacia mixture alone. Plotting the volume-flow on the ordinates against driving pressure on the abscissas (fig. 12) we get curves showing a deviation from the straight line. In some instances the deviation is relatively slight, but frequently results shown in figure 12 are obtained. Oedema of the organs was prevented

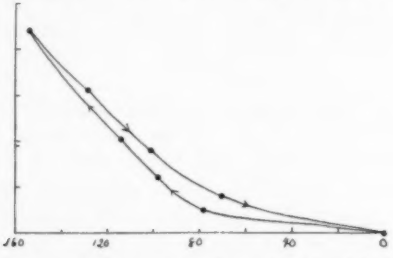

Fig. 12. Perfusion flow of gum acacia through a dead kidney plotted on the ordinates against the head of pressure on the abscissas. The lower curve was obtained by increasing and the upper by decreasing the head of pressure.

by the employment of acacia. But to rule out this factor completely successive curves of volume-flow were plotted, first with a rising pressure and next with falling pressure. The curves are similar and bear a resemblance to the curves obtained in haemorrhage in the living animal. There are, however, two important differences. The initial decrease in flow is not as rapid as in haemorrhage and the plateau near its beginning points low, that is to the intersection of the ordinates and abscissas at zero. The curve suggests that as driving pressure increases, the vessels stretch which produces an increase in the effectiveness of the driving pressure obtaining. That most of this stretching occurs only after a relatively high pressure (in fig. 12, about 90 mm. Hg.) is reached, is a factor of considerable practical importance in the treatment of lowered blood pressure from haemorrhage and tissue abuse.

That this stretching of vessels in dead organs actually occurs was shown by perfusion with a suspension of blood corpuscles. The percentage of corpuscles of the "venous blood," and it might be stated that the venous flow was slow, fluctuated with the head of pressure. (See table 3.) Apparently the lower perfusion pressures are not sufficient to stretch the vessels or deform the corpuscles sufficiently to permit their free passage and filtration of the blood occurs.

The finding of Denning and Watson (29) that suspended particles increase the deflection of the curve of flow was confirmed by comparing curves of flow obtained with 7 per cent gum acacia suspension alone and a mixture of blood and gum acacia.

It is of considerable interest to know how much the corpuscular factor determines volume-flow in living tissue. If the high peripheral red count noted by Cannon, Fraser and Hooper (30) represents the count of arteriole blood it might indicate a constriction sufficient to

TABLE 3

OBSERVATION	BLOOD PRESSURE	RATE OF PERFUSION	PER CENT OF RED CELL IN "VENOUS BLOOD"
1	62	4.6	9.4
2	120	13.8	14.0
3	160	19.5	16.4
4	118	5.4	11.1
5	90	2.6	8.7

block the passage of red cells as they suggested. That such constriction may occur was also pointed out by Cohnstein and Zuntz (31). The lower red counts of venous blood coming from a constricted area, noted by Sherrington and Copeman (32) would also indicate that a constriction may produce a partial filtration of the blood.

The effect of acid injection on the height of the plateau of the curve of volume-flow. From the experiments on perfusion of dead organs it is apparent that purely mechanical factors independent of physiological reactions account in part for the contour of the volume-flow curve obtained from the living animal. Two differences in the curves, however, were noted, one, the lesser deflection in the first segment and the other, the pointing of the plateau to zero on the abscissas. In the living tissue the plateau points well beyond this point, showing definitely that in haemorrhage in addition to the checking of constriction, we have a vascular dilatation accounting for the second deflection of the curve.

Pilcher and Sollmann (33) observed that the vasoconstriction in the early part of haemorrhage merged at a mean blood pressure of 90 to 100 mm. Hg. into a period of dilatation of central origin. The question in which we are interested here is whether this dilatation may be augmented by peripheral chemical dilatation from concentration of metabolites.

It is fully realized that substances other than carbon dioxide may act as chemical regulators of volume-flow, yet the results of experiment 77 suggested experiments along this line. It was hoped that by decreasing the CO_2 capacity of the blood by acid injection the effectiveness of the CO_2 added to the blood as it passes through the tissues would be increased and be demonstrated by dilatation. If this occurs a reduction of alkaline reserve prior to bleeding should elevate the plateau. Variable results were obtained and were attributed to the fact that a reduction in alkaline reserve may increase the effectiveness of CO_2 stimulation of the vasoconstrictor center as well the peripheral mechanism.

The relation of alkaline reserve to superbasal-flow of blood. In the case of superbasal-flow, however, the effects of lowered alkaline reserve might be studied to better advantage for the reason that conditions are more local, the accessions of CO_2 to the blood passing through an activated gland being greater than the accession of CO_2 to the blood passing through the resting vasoconstrictor center. In some experiments acid injection did increase the superbasal-flow. Though a uniform demonstration would be more convincing yet I feel that the greater hydrogen ion concentration established in blood poor in reserve alkalinity as it passes through an activated tissue might be a factor in securing adequate flow and protecting the tissue against still greater acid reactions.

The relation of alkaline reserve to volume-flow is a perplexing one. The double effect of increased hydrogen ion concentration is easily demonstrated. But the question is, can changes in hydrogen ion concentration be looked upon as a normal mechanism of volume-flow control and does the factor enter into play in conditions of lowered blood pressure preceding the marked reaction just prior to death? The problem seems to call for a quantitative study of the threshold and of the difference in threshold of stimulation for the central and peripheral mechanisms.

SUMMARY

This work represents an attempt to obtain data for the interpretation of electrical deflections in the salivary gland by determining the factors controlling the volume-flow of blood and the effects of decreased flow on metabolism.

The experiments were performed on the submaxillary gland of the dog.

By measuring the volume-flow of blood during prolonged chorda-stimulation it was found that the synchronous fluctuations in secretion and electrical deflection were accompanied by parallel changes in volume-flow of blood.

These observations raised the question of the interdependence of volume-flow of blood and tissue metabolism,—are changes in the flow primary, causing fluctuations in secretion or does increased secretion call forth the augmented flow of blood?

The possibility of the secretory fluctuations being an inhibitory phenomenon is discussed.

That the changes in volume-flow of blood exceed the variation in secretion is pointed out; also that fluctuations in volume-flow of blood with chorda stimulation may occur in the absence of visible secretion.

The change in volume-flow of blood with prolonged chorda stimulation is, therefore, a vasomotor phenomenon—either of metabolite or of vasomotor nerve origin.

The measurement of the latent period of secretion and of vasodilatation, the selective paralyzing of the secretory fibers with atropin and the selective stimulation by grading of intensity failed to give conclusive evidence of two independent sets of fibers—vasomotor and secretory.

For that reason the problem was approached by a number of indirect methods which by cumulative evidence might suggest the mechanism of volume-flow control.

Methods were chosen which would yield data of theoretical interest and also of practical value in connection with conditions of lowered blood pressure.

The observations of Barcroft and of Carlson of increased flow of blood elicited by sympathetic stimulation in the cat were confirmed in a few instances in the dog.

Combined stimulation of the chorda tympani and vago-sympathetic may elicit a summation of secretion accompanied by a flow of blood less than that obtaining during chorda stimulation alone.

Since increased volume-flow of blood can occur only through inhibition of the constrictor tonus, a constant potential tonus is required to study the relation of metabolism to volume-flow of blood.

This relation was studied by eliciting progressively increasing amounts of saliva by chorda stimulation during a constant head of pressure and measuring the extra flow of blood.

By plotting the superbasal-flow of blood on the ordinates against metabolism on the abscissas we get a straight line. This suggests a purposive mechanism of volume-flow control though not disproving the theory of nerve control. The apparently perfect adjustment of flow to metabolism might point more strongly to metabolite control.

By studying the time relation of superbasal flow to superbasal metabolism at various heads of pressure, it appears that some of the increased flow of blood is not accounted for by the action of dilator nerves.

The failure of metabolites to counteract more fully the effects of falling pressure may be attributed to the fact that a greater constrictor tonus must be inhibited and that the head of pressure obtaining is insufficient to take advantage of the inhibition produced.

By recording the flow of blood through a resting gland with a decreasing head of pressure suggestions on the relation of basal flow to basal metabolism were obtained.

The sudden decrease in flow giving way to a nearly constant or even accelerated flow with a further fall in pressure supported the working hypothesis—that the flow of blood might decrease to a point permitting a concentration of metabolites in the tissues and tissue blood sufficient to produce a dilatation, thereby tending to maintain a constant flow of blood despite a further fall in pressure.

The importance, however, of a decrease in central constrictor-tonus is evident.

To determine the relative effects of failure of the vasomotor center and the concentration of metabolites curves of flow from glands with the vago-sympathetic intact and severed were compared.

According to the law of Poiseuille, if the factors of caliber and viscosity remain constant the curve of flow plotted against head of pressure is represented by a straight line.

Deflections of this curve from the straight line in the course of haemorrhage must therefore be due to changes either in caliber or in viscosity.

The factor of viscosity has relatively little effect on the general contour of the curve but rather on its position.

The large deflections are, therefore, mainly a function of changes in caliber.

Since in the early stages of haemorrhage there may be a rise of pressure accompanied by a marked decrease in volume-flow the steepness of the first segment of the curve is largely attributable to a vasoconstriction of central origin.

But after vago-section the initial fall in pressure is likewise accompanied by the greatest decrease in volume-flow, suggesting that with the vagus intact both passive constriction and active constriction of central origin occur.

The gradients of the second as well as the first segment of curves with vagi intact and severed are somewhat comparable. The third segments, however, show a marked divergence.

The fact that at low pressures a further decrease in pressure is accompanied on the intact side by a suddenly decreased, and on the severed side by a suddenly increased flow, suggests the possibility of a central and peripheral action of increased concentration of CO_2.

As a control involving only mechanical factors the volume-flow through dead organs with varying heads of pressure was determined.

The presence of the first deflection of the curve shows the occurrence of passive constriction and the importance of a high stretching head of pressure.

The pointing of the plateau to zero on the abscissas, which is not the case in living tissue, shows that in haemorrhage after active and passive constriction have ceased dilatation begins.

The relative importance of failure of the vasomotor center and of concentration of metabolites in elevating the plateau is difficult to determine.

An attempt to raise the plateau by a reduction of the alkaline reserve previous to a lowering of the head of pressure proved unsuccessful, but that the superbasal-flow of blood may be augmented by increasing the effectiveness of CO_2 liberated in metabolism is suggested.

As to the existence of vasodilator nerves, the question which initiated this research, nothing definite can be said.

We have no proof that such nerves do not exist, neither have we proof that metabolites cannot adequately control the volume-flow of blood.

All that can be said at present is that if dilator nerves do control the flow of blood this flow may be augmented still more by an accumula-

tion of metabolites. Many of the observations might apply to both theories, some, however, seem to point more strongly to the theory of metabolite control.

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STUDIES ON THE SUBMAXILLARY GLAND

IV. A Comparison of the Effects of Hemorrhage and of Tissue-Abuse in Relation to Secondary Shock[1]

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This research deals with circulatory and nutritional disturbances resulting from hemorrhage, from tissue-abuse and from acid injection. It is in part an application of the results outlined in the preceding paper as well as a presentation of additional data.

The term shock has been applied to a variety of conditions of lowered blood pressure regardless of whether the condition is attributable to a very sudden disturbance resembling, for example, the immediate inhibition of reflexes following transection of the cord, or associated with a relatively slow and progressive nutritional disturbance accompanying a progressive fall of blood pressure. Attempts have been made to differentiate between disturbances of sudden and more gradual onset by Cobbett (1), Lazarus-Barlow (2) and others and designated as shock and collapse respectively. Cannon, Cowell, Fraser and Hooper (3) divide these conditions of lowered blood pressure into primary and secondary shock. "Primary shock may come on so soon after injury as to be accounted for only as a result of nervous action" while secondary shock results after prolonged effects of inflicted injury, hemorrhage, exposure, etc. This research deals with secondary shock alone.

Shock has been defined as a disturbance in which the blood pressure has reached a level of about 50 mm. Hg. and has thus led more to work on the relation of blood pressure to the condition than on the relation of volume-flow of blood to the general nutrition obtaining.

[1] Reported in part before the American Physiological Society, Proceedings in this Journal, 1918, xlv, 545.

Volume-flow of blood appeared to be the more fundamental problem and as the results will show mean blood pressure gives very little evidence of the gravity of the disturbance. A primary rise in pressure occurring both in hemorrhage and tissue-abuse may be accompanied by a large decrease in volume-flow of blood, constituting the greater part of the total decrease reached by a subsequent fall to zero pressure.

Shock in this research is looked upon much as outlined in a previous report (4). It is considered in a very general way as a combined circulatory and nutritional disturbance resulting from a number of forms of tissue-abuse which if left to run their course may lead to death. Even though these forms of tissue-abuse, the initiating causes, may be removed the fatal issue is not necessarily avoided. Some changes apparently have taken place as a result of the tissue-abuse which sustains the condition. The factors in shock are therefore divided into *initiating factors* and *sustaining factors.*

The definition given by Lusk (5) explains what is meant by nutritional disturbance. "Nutrition may be defined as the sum of the processes concerned in the growth, maintenance and repair of the living body as a whole or of its constituent organs." The definition is accepted in this paper in its broadest sense.

Concerning the initiating and sustaining factors in shock, we know that experimentally a condition apparently comparable to shock as recognized clinically can be produced by numerous procedures. Shock is produced by mechanically inflicted injury, such as manipulation of the intestines or the crushing of muscle, by the effects of cold produced by immersion of the animal in iced water or the introduction of ice into the abdominal cavity, by burns or scalds, by over-anesthesia, by dehydration elicited by the introduction of hypertonic solutions into the abdominal cavity, by interference of the blood supply to the tissues accomplished by clamping of the inferior vena cava, clamping of the aorta and ligation of the veins from the extremities, by limitation of the oxygen intake, etc. For that reason forms of tissue-abuse which may be effective in producing shock are designated as initiating factors.

These initiating factors ultimately lead to changes in the tissues which have their influence on the "sum of the processes concerned in maintenance and repair" of living tissue. There is cellular damage, increased permeability of cells, transudation, eventual decrease in blood volume, slowing of the blood stream, etc. These represent sustaining factors some of which are not readily removed.

Since shock as recognized clinically is associated with mechanically inflicted injury, surgical or otherwise, it might be well to designate this injury as the *primary initiating factor* and other initiating factors which aggravate the condition, whether they were in operation before or after the inflicted injury, as *accessory initiating factors*. To the list should be added any form of tissue-abuse even though it alone may not, in the ordinary sense of the term, produce shock, e.g., thirst, fatigue, hunger, etc.

When the condition of shock is recognized at the front, the circulatory disturbances have reached a serious stage. Hence an important phase of the problem of shock is a matter of obtaining a basis for combating these circulatory disturbances.

There appears, however, to be another phase of equal if not greater importance, and that is the means of preventing these disturbances, a means of detecting the seriousness (6), (7), (8), of the initiating causes before the outstanding symptom of lowered blood pressure has developed, before serious structural changes have occurred and before irreparable damage has been done.

The emphasis laid on blood pressure previously expressed itself in the employment of pressor substances such as adrenin and pituitrin for the elevation of pressure at the expense of volume-flow. Though these forms of treatment have been largely discarded it appears that too much weight is still given to the significance of mean blood pressure relative to the conditions obtaining. Here lies a danger of permitting the development of shock which might otherwise be avoided. Although the animal itself employs pressor methods to combat a lowering of pressure, results have definitely indicated that a part of the problem of treatment and prevention of shock lies in the combating of these pressor reactions of the animal.

Though a blood pressure of normal magnitude is essential under normal conditions to maintain proper nutrition, and though blood pressure per se may be a factor of importance in the physiology of secretion, lymph, etc., yet we know that conditions of lowered blood pressure produced by transection of the cervical cord are survived. The survival is probably due to the fact that the lowered blood pressure is a result of vasodilatation, permitting an adequate volume-flow of blood.

Though the study of shock is at present receiving more attention, the common observation that "hemorrhage is the most potent shock producing factor" calls for a close comparison of the two conditions.

Hemorrhage and shock have many points of similarity. It appeared to me that the study of hemorrhage was important not only in itself, but especially in its relation to shock.

To be sure, certain outstanding features of the two conditions should call for different forms of treatment, but if these conditions are found closely comparable, if factors controlling the volume-flow of blood operate in a similar fashion, there might be a common basis for treatment and we would have at our disposal an easy and perfectly controllable method of abusing tissue and producing shocklike conditions amenable to the study of blood substitutes and requiring a minimum amount of aseptic surgery.

In these experiments shock was produced by exposure and manipulation of the abdominal viscera.

The data deal with:

a. Basal and superbasal-flow of blood in relation to metabolism.

b. The dependence of normal metabolism on basal blood-flow as measured by decreased alkaline reserve, etc.

c. A comparison of the basal-flow accompanying decreased blood pressure produced by hemorrhage and by tissue-abuse.

d. A comparison of the superbasal-flow of blood obtaining with lowered pressure from hemorrhage and tissue-abuse.

e. The relation of alkaline reserve to volume-flow of blood in hemorrhage, tissue-abuse and acid injection.

f. The respiratory function during hemorrhage, tissue-abuse and acid injection.

g. Comparative effects of gum acacia injection on the basal-flow of blood after hemorrhage and tissue-abuse.

The relative importance of the sustaining factor of slowed circulation depends on the degree to which tissues withstand a reduction in volume-flow of blood without injury. The detrimental effects of a marked decrease in flow accompanying a fall in pressure are easily demonstrable, but the effects of a smaller decrease in volume-flow unaccompanied by a change in pressure are not so apparent. Thus a demonstration of the dependence of normal metabolism on a normal flow of blood is desirable.

Data bearing on this point were obtained from experiments in this and the preceding paper (9) on *a*, the dependence of volume-flow of blood on metabolism; and on *b*, the dependence of metabolism on the volume-flow of blood.

a. Basal and superbasal-flow of blood as affected by metabolism. If we accept the view that liberation of metabolites increases the flow of blood on tissue activation, the experiments of the preceding paper show that the mechanism of volume-flow of blood is finely adjusted to metabolism and suggest that a normally increased function requires an increased volume-flow. It will be recalled that a stimulus too weak to elicit a visible secretion but strong enough to produce an electrical deflection, is accompanied by a considerable increase in volume-flow of blood. The linear relation of superbasal-flow to superbasal-metabolism whether dependent on the action of dilator fibers or dilator metabolites suggests a purposive mechanism of volume-flow control. These observations along with others, e.g., the slower recovery of a gland from activation when a low blood pressure prevails, the after-flow of blood following the release of arterial obstruction, etc., all point to a relatively narrow margin of safety of decrease in volume-flow.

b. Dependence of the tissues on basal-flow of blood as measured by decreased alkaline reserve. The experiments cited on the relation of volume-flow of blood to metabolism suggest a close dependence of the tissues on volume-flow, but more direct experiments were desirable. Knowing that a decreased oxygen supply to the tissues leads to the formation of acids other than carbonic acid and that these acids reduce the alkaline reserve, we might follow in a quantitative way the extent to which decreased volume-flow of blood affects oxidations.

Samples of blood were taken at regular intervals—first, during a normal control period with a constant volume-flow; second, during a period of reduced flow produced by hemorrhage; third, during another period of further reduced flow from a second hemorrhage. (See fig. 1.) The alkaline reserve of the blood was determined with the Van Slyke method (10) at forty-five minute intervals and plotted on the ordinates against time on the abscissas.

During the period of normal volume-flow the alkaline reserve remains approximately constant. During the next two periods, initiated by hemorrhage, the gradient takes two sharp dips, indicating that the disturbance in oxidations varies directly with the decrease in volume-flow of blood.

Objection to this conclusion might be raised on the ground that the decrease in alkaline reserve is attributable to a dilution of the blood by tissue fluids poor in alkali (11). To determine the importance of this factor in the dog the percentage of hemoglobin was likewise plotted.

The curve shows the effect of dilution, assuming the tissue fluids entering the blood to be free from alkaline reserve. The relative dipping of the two gradients therefore, indicates that the dipping of the CO_2 gradient is to be attributed mainly to decreased oxidations. This becomes more apparent through the observation that a decreased alkaline reserve of the blood is partially compensated by attraction of tissue alkaline reserve into the blood. (See page 482.) The degree to which this occurs may possibly account for the variability noted in the configuration of the curves, for all the curves do not show the sharp bending of the gradient or the perfect alignment of observations noted in figure 1. The attraction of alkaline reserve into the blood produces a bending of the curve upward and partly conceals the effects of decreased volume-flow of blood.

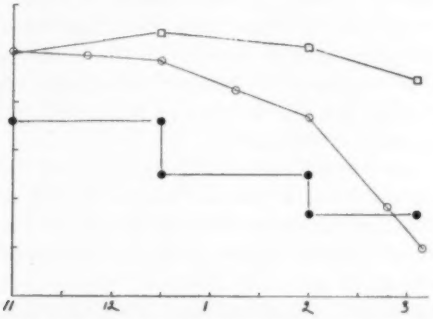

Fig. 1. Curves showing the relation of oxidative processes to volume-flow of blood. Hemoglobin percentage, carbon dioxide capacity and blood flow plotted on the ordinates against time on the abscissas.

Upper curve, hemoglobin percentage; middle curve, carbon dioxide capacity; lower curve, volume-flow of blood.

The detrimental effects of decreased flow are also shown in figures 3 and 4 where the variability of the factor of safety becomes evident. Take, for example, experiments 87 and 103 in which the volume-flows are markedly reduced, but apparently well borne as indicated by the horizontal CO_2 gradient. The interesting feature is that when the break in the CO_2 and respiratory gradients occurs it is abrupt. In experiments 85, 99 and 102, the deflection of the CO_2 and respiratory gradients follows promptly the decrease in volume-flow indicating an exceedingly small factor of safety. The experiments apparently represent conditions favorable for the development of shock.

c. A comparison of basal-flow of blood accompanying decreased blood pressure produced by hemorrhage and by tissue-abuse. If oxidations are as closely dependent on volume-flow as these experiments indicate, a reliable index to the flow of blood in shocklike conditions is desirable. The criterion most commonly used for gauging the circulatory disturbance in experimental shock is mean blood pressure. As was shown in the preceding paper, mean blood pressure in itself gives very little information on the volume-flow of blood obtaining. Unless blood pressure per se is of considerable importance in functions other than volume-flow of blood, it is a very misleading criterion of the condition of the animal.

The danger of gauging volume-flow of blood by mean blood pressure alone is due mainly to the double relation of pressure to the caliber of vessels—a decrease in caliber may be the cause of a rise in pressure and a fall in pressure may be the cause of a decrease in caliber. At least we know that in the submaxillary gland with the vago-sympathetic cut, vasoconstriction accompanies the primary fall in blood pressure. Perfusion of dead organs likewise shows a passive constriction during the early decrease in head of pressure.

Note that in figure 4, experiment 98 and figure 7, experiment 40, a large decrease in volume-flow of blood occurs during a perfectly stationary blood pressure. In experiment 87, figure 4 and experiment 85, figure 3, hemorrhage initiates a rise of blood pressure probably due to an over-constriction.

In figure 4, experiment 87, hemorrhage produced a rise in mean blood pressure from 112 to 116 mm. Hg. and decreased the volume-flow of blood 61.6 per cent of the original flow. The further fall from the augmented pressure of 116 mm. to 50 mm. Hg. produced a relatively slow decrease in volume-flow, only 17.8 per cent of the initial flow.

In figure 3, experiment 85, the rise in pressure is greater (11 mm., from 116 to 127 mm. Hg.) and the decrease in volume-flow is likewise greater —65 per cent. A point of special interest in this experiment is the sudden checking of the decrease in volume-flow during the rise in pressure which is changed to an increased flow during a fall in pressure.

The practical significance of mean blood pressure[2] as a gauge to the general condition is apparent: a constantly maintained pressure may be accompanied by a large decrease in volume-flow; two identical pressures may occur at different stages, one representing normal flow

[2] The relation of pulse pressure to the circulatory disturbance was not studied.

and the other a marked circulatory disturbance; a rise in pressure may be accompanied by a decrease in volume-flow of blood from two to three times as great as that accompanying the subsequent fall to zero pressure. The important point common to all observations is that the greatest decrease in volume-flow of blood occurs during a relatively small deviation from the initial pressure and that subsequent to this sudden decrease in volume-flow a further decrease in pressure of 80 mm. Hg. or more may be accompanied by only a relatively small decrease, no decrease or even an increase in volume-flow of blood.

The circulatory disturbances, so far as the general volume-flow of blood is concerned, may be potentially as bad with a high as with a low blood pressure. The main difference in the two conditions is that the high pressure may represent a disturbance of a shorter duration and for that reason be more amenable to treatment. But in so far as the brain and heart consume more oxygen than the other tissues, and in so far as vasoconstriction of central origin may not reduce the flow of blood through these structures, as through other structures, the maintenance of a high blood pressure may be of some significance.

Usually the large and sudden decrease in volume-flow is reached before the pressure has fallen to a level of 90 mm. Hg. In one experiment on record, hemorrhage raised the initial pressure from 130 mm. to 140 mm. Hg. and when the initial pressure was subsequently reached the volume-flow was decreased 78 per cent. Therefore, pressures from about 140 or even higher to 90 mm. Hg. may be of little value regarding the volume-flow of blood and the general condition obtaining unless a previous record of pressure is at hand.

Figures 3, 4 and 7 show the similarity of the curves resulting from hemorrhage and from tissue-abuse. It appears, therefore, that fundamentally the same factors controlling volume-flow of blood are operating in the two conditions. These factors have been considered in some detail in the preceding paper on volume-flow of blood (12).

The well-known active constriction in hemorrhage may be looked upon as a reaction to decreased blood volume. Pilcher and Sollmann (13) refer the constriction to decreased oxidations in the vasomotor center resulting from the decreased volume. I am inclined to feel that the reaction may be of the nature of a volume-reflex similar to the lowering of blood pressure through the depressor nerve. This is suggested by the fact that although large and even rapid hemorrhages may occur without a lowering of the pressure yet constriction results. But this is not the question in which we are interested at present.

Protocols of several experiments are given to show the effects of hemorrhage. What concerns us in these protocols is not the maximum amount of bleeding a normal individual may withstand and still recover but rather the minimum amount producing a decided decrease in volume-flow of blood. These data are needed to ascertain whether we may have serious constriction of a similar source from transudation in tissue-abuse and to determine the relative importance of a decrease in blood volume whether it arises from hemorrhage or transudation both in initiating and sustaining shock.

Experiment 85. Repeated hemorrhages.
Weight of dog, 12,000 grams.
Blood volume, 1,080 cc.
No. 5. Total amount bled, 100 cc., about 9.3 per cent of blood volume. Total reduction of volume-flow, 49 per cent.
No. 9. Total amount bled, 195 cc., about 18.0 per cent of blood volume. Total reduction of volume-flow, 65 per cent.
Experiment 87. Repeated hemorrhages.
Weight of dog, 14,940 grams.
Blood volume, 1,340 cc.
No. 3. Total amount bled, 115 cc., about 8.6 per cent of blood volume. Reduction of volume-flow, 61.6 per cent.
Experiment 103. Repeated hemorrhages.
Weight of dog, 18,300 grams.
Blood volume, 1,675 cc.
No. 5. Total amount bled, 175 cc., about 9.6 per cent of blood volume. Total reduction of volume-flow, 42.2 per cent.
No. 7. Total amount bled, 275 cc., about 15 per cent of blood volume. Total reduction of volume-flow, 44.8 per cent.
Experiment 46. Repeated hemorrhages.
Weight of dog, 15,800 grams.
Blood volume, 1,420 cc.
Total amount bled, 150 cc., about 10.5 per cent of blood volume. Total amount bled, 360 cc., about 25 per cent of blood volume.
Total reduction of volume-flow, 69.5 per cent.
Experiment 49. Continuous hemorrhage through the vessel of the gland.
Weight of dog, 13,250 grams.
Blood volume, 1,196 cc.
Total amount bled, 539 cc., about 45 per cent of blood volume.
Reduction of volume-flow, 70 per cent.
Experiment 48. Continuous hemorrhage through the vessels of the gland.
Weight of dog, 12,590 grams.
Blood volume, 1,135 cc.
Total amount bled, 325 cc., about 28 per cent of blood volume.
Total reduction of volume-flow, 42 per cent.
Total amount bled, 474 cc., about 42 per cent of blood volume.
Total reduction of volume-flow, 66 per cent.

Observe the varying effects of hemorrhage. The smaller decrease in volume-flow elicited by a continuous hemorrhage agrees with the observation that animals withstand slow hemorrhage better than more rapid hemorrhage. But another explanation is possible. The relative and absolute decrease in volume-flow resulting from reduced blood volume depends upon the constrictor tonus at the beginning of the experiment. If for any reason that is high, (from previous concentration, for example) the effects of lost volume are reduced. For a specific case refer to experiment 100, page 478. Had the initial determination of volume-flow been postponed but a few minutes the major effects of decreased volume would have been missed. It is probable that in most cases the volume-flow decreased more during the course of the experiment than is represented in the protocols and graphs. But even disregarding such possibilities the percentage decrease of volume-flow of blood far exceeds the percentage decrease in blood volume in every experiment. The maintenance of a normal blood volume is, therefore, an extremely important function.

The question arises—does the same mechanism reduce volume-flow in tissue-abuse?

From the observations of Sherrington and Copeman (14), Cobbett (15), Lazarus-Barlow (16), Vale (17), Gasser, Meek and Erlanger (18) and others we know that concentration of red cells in the blood is a striking feature of tissue-abuse. Occasionally, even in the early periods of an experiment I have noted a diminution in blood volume from this source amounting to more than 20 per cent. Cobbett records a reduction of 30 per cent. Employing the gum acacia method of Meek and Gasser (19) for determining blood volume, Gasser, Meek and Erlanger find an average reduction of blood volume amounting to 17.6 per cent. The decrease of volume-flow in tissue-abuse is, therefore, readily accounted for in most instances.

Though the vasoconstrictor center is very sensitive to small changes in blood volume, slight reduction in the early stages of tissue-abuse might appear insufficient to elicit the decreased volume-flow. The experiments of Pilcher and Sollmann showing that a shunting of blood to a local area by local dilatation produces a general vasoconstriction meets this difficulty for the viscera, after manipulation, give every appearance of acute inflammation with an increased blood flow. The effects on constriction of decreased circulating blood from stasis should also be mentioned (Welch, Mann, and Erlanger and Gasser).

Only rarely can the effect of reduced blood volume from tissue-abuse be studied as clearly as in hemorrhage. Therefore experiment 100 is described in some detail, an experiment in which for some unknown reason rapid and marked concentration of the blood occurred with the animal almost intact even before the abdomen was opened. The animal experimented on was a male bull dog apparently in perfect health, previously injected with morphine sulphate which had taken marked effect. The dissection about the submaxillary gland was possible without the use of ether.

The volume-flow of blood was 100 drops per minute and the volume percentage of red cells was 60. To eliminate effects other than manipulation of the viscera, which was to follow, another determination of the blood flow was made one half-hour later and found to be 30 drops per minute Not knowing how to account for such a striking reduction in volume-flow, the blood pressure having remained constant at 130 mm. Hg. and the dissection of the gland having been a very easy one, the percentage of red cells was again determined and found to be 79. The diminution in blood volume which such concentration entails accounts for the serious constriction. That such disturbances occur without any outward signs whatever suggests an explanation of some of the more mysterious cases of shock. The experiment shows again the danger of relying too much upon blood pressure measurements.

That general constriction rather than exhaustion of the vasomotor center exists in the early stages of shock is generally accepted, (20), (21), (22), (23), (24), (25), (26), but experiments such as no. 100 point definitely to the source of the reaction—a decreased blood volume similar to the source of constriction in hemorrhage.

Epstein (27) calls attention to concentration of red cells from administration of ether. Whether the small amount used in experiment 100 could account for the transudation of plasma is questionable, however.

It might be mentioned in connection with decreased blood volume that manipulation of the intestines in many animals produces a prompt and visible thickening of the intestinal wall and cessation of manipulation is frequently followed by considerable recovery of the normal thickness. These events may be frequently repeated; and they presumably are accompanied by fluctuations in blood volume as is indicated by synchronous oscillations in mean blood pressure.

I also have observed that manipulation of limited segments of the intestines though initially leading to a definite fall in pressure soon becomes less effective. This may be due to the fact that maximum

transudation in the manipulated segment has occurred for manipulation of a new segment now leads to further transudation and fall in pressure. Other explanations of course are possible.

All the experiments indicate the similarity of the vascular reactions in hemorrhage and tissue-abuse. This is borne out by the following observations. Pilcher and Sollmann noted that animals with a low initial pressure withstand hemorrhage far better than those with a high pressure, a fact also noted in the present research. The manner in which some animals withstand large and even rapid hemorrhages without a lowering of the pressure during bleeding is remarkable. The counterpart of this is the observation of Erlanger and Gasser that animals with a high initial pressure respond less well to treatment after clamping of the cava than do animals with a lower initial pressure.

d. A comparison of the superbasal-flow of blood with lowered blood pressure from hemorrhage and tissue-abuse. In the regulation of volume-flow of blood to the tissue needs we are not only interested in the manner in which the flow to a resting tissue varies with changing head of pressure, but also in the superbasal-flow for activated tissue. Undoubtedly in the marked nutritional disturbances occurring in conditions of lowered blood pressure, tissues suffer not only from decreased basal-flow but likewise decreased superbasal-flow. That the liver, kidney and probably the heart are called upon for increased activity seems possible.

By stimulating the chorda tympani so as to elicit equal amounts of saliva with varying heads of pressure and measuring the extra flow of blood per drop of saliva we determined the conditions under which tissues work in hemorrhage. (See page 451, paper III) (28). It was found that superbasal-flow divided by superbasal-metabolism decreases out of all proportion to the decrease in pressure.

The effects of tissue-abuse are equally as great as hemorrhage (see fig. 2 and table 1). In figure 2 the basal-flow of blood is plotted against mean blood pressure in the usual way, and observations shown in the table are designated on the curve.

The initial changes in blood pressure have the most detrimental effects on superbasal-flow as well as on basal-flow. Though a 100 mm. Hg. might be considered a normal pressure for some dogs, yet when that pressure is reached in this experiment the superbasal-flow of blood is more than halved. Since the stretching head of pressure seems adequate to take advantage of any inhibition of tonus and produce an adequate superbasal-flow of blood we are reminded of the

experiments of the preceding paper of combined and separate stimulation of the chorda tympani and vago-sympathetic (see fig. 4, paper III).

The amount of secretion obtained by combined stimulation is greater than that obtained by chorda stimulation alone, and yet is accompanied by a smaller volume-flow of blood. This smaller volume-flow

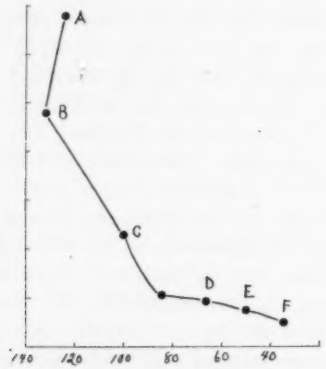

Fig. 2. Basal-flow of blood, from an experiment on the effects of tissue-abuse, plotted on the ordinates against mean blood pressure on the abscissas.

TABLE 1

OBSERVATION	BLOOD PRESSURE IN MM. HG.	BASAL FLOW OF BLOOD IN DROPS PER MINUTE	SECRETORY BLOOD FLOW DIVIDED BY SECRETION
A	124	68.0	13.9
B	132	48.0	12.0
C	100	23.0	5.9
D	66	9.5	3.3
E	50	7.7	1.8
F	35	5.0	0.0

was attributed to the failure of the chorda effects to inhibit the increased tonus resulting from vago-sympathetic stimulation.

During the earlier stages of hemorrhage and tissue-abuse we have comparable conditions; an increased constrictor tonus which must be inhibited before a superbasal-flow of blood can take place. That this is a factor of some significance is indicated in figure 2 and table 1, for despite the increased stretching head of pressure at *B*, the increased

constrictor tonus which is the cause of the rise in pressure is not as thoroughly inhibited and the superbasal-flow of blood is decreased.

The danger that such a mechanism may act detrimentally on some important activated tissue is apparent.

e. The relation of alkaline reserve to volume-flow of blood in hemorrhage, tissue-abuse and acid-injection. The relation of volume-flow of blood to alkaline reserve is a perplexing one.

It will be recalled (29) that with innervation intact acid injection may decrease the basal-flow of blood and with the vago-sympathetic severed elicit increased flow; that the superbasal-flow of blood appears at times to be increased by a decreased alkaline reserve, and that a possibility of a balanced action, peripheral and central exists.

From a practical point of view, I have nothing definite to add but some observations may be of significance.

Animals withstand large injections of acid which may lead to an increased rather than decreased pressure. An increased pressure of 140 mm. Hg. at a Van Slyke reading of 6 giving way suddenly to zero pressure on further injections indicates that the heart may for a time at least execute extra work with an exceedingly low alkaline reserve.

But that the organism strives to prevent a development of a low alkaline reserve long before that is reached is shown in table 2; n/2 hydrochloric acid was injected intravenously at intervals of approximately 30 minutes and the CO_2 content followed. (This experiment was performed for another purpose, therefore the intervals and amount of acid are not the same. The results, however, are so striking that the conclusions are not affected.) The CO_2 content was determined prior to each injection. The reduction of alkaline reserve with each injection divided by the amount of acid injected gives the effectiveness of the acid to neutralize the reserve at different stages of the experiment. The effectiveness rapidly decreases and at the end the tissue reserve enters the blood so rapidly that the acid injection in the interval allowed is without effect.

The wide variations of alkaline reserve noted by Henderson and Haggard (30), and by Scott (31) and their relation to morphinization were observed by me. In addition it was found that the lethal injection of acid per kilo of body weight was less in animals with a high alkaline reserve than in animals with a low alkaline reserve.

The passage of alkaline reserve into the blood may represent one of the normal mechanisms of combatting increased hydrogen ion concentration yet as Bayliss (32) pointed out a low alkaline reserve may

not be without its advantages when the effects on volume-flow of blood, gaseous exchange in the lungs and in the tissues in general are considered. Though the results of the preceding paper suggested similar ideas, a maintenance of normal alkaline reserve is probably preferable. Erlanger and Gasser find that injection of carbonate in concentrated form is distinctly harmful but is not harmful when injected in weaker concentrations. In the matter of treatment Bayliss and Erlanger and Gasser feel that with improved circulation the alkaline reserve will take care of itself. The rapid passage of alkaline reserve into the blood as shown in table 2 illustrates how readily a lowered alkaline

TABLE 2

$$\frac{\text{Van Slyke Readings}}{\text{Acid injected}} = \text{Effectiveness of acid to reduce the alkaline reserve}$$

$$\frac{51.8 - 42.4}{26} = 0.361$$

$$\frac{42.4 - 28.0}{50} = 0.288$$

$$\frac{28.0 - 20.0}{28} = 0.285$$

$$\frac{20.0 - 14.0}{30} = 0.200$$

$$\frac{14.0 - 9.7}{32} = 0.134$$

$$\frac{9.7 - 9.7}{19} = 0.000$$

reserve is overcome. The work of Howell, Seelig, Mann and others, however, indicates the beneficial effects of injection of carbonate.

f. The respiratory function during hemorrhage, tissue-abuse and acid injection. The similar and normal constrictor reaction of the vasomotor center in hemorrhage and tissue-abuse and the normal reaction of the cardio-inhibitory center in shock (33), suggested also the need of a closer comparison of the respiratory function in the two conditions than has hitherto been made. In such a study we are interested in the reaction of the respiratory center to the decreasing alkaline reserve prevailing in order to determine more nearly the relative

importance of a general involvement of the medullary centers and the relation of the reaction to general oxidative processes.

In studying respiration we must distinguish between cause and effect. Van Slyke (34), Henderson and Haggard, Scott and others point to the readiness with which the alkaline reserve of the blood may shift. I have found the effects of morphinization pointed out by Henderson and Haggard and Scott to be striking and also that painful stimulation from tissue-abuse is likely to lead to over-ventilation with a reduction of the alkaline reserve of the blood.

It is therefore realized that a study of respiration is best carried out on a decerebrate animal. But the curves will show that under certain circumstances, with both acid injection and hemorrhage, a uniformity of results is obtainable. In tissue-abuse where painful stimuli enter into play such uniformity is not to be expected and therefore if only occasional experiments are comparable to those of hemorrhage we might be justified in concluding that the response to changing alkaline reserve would be similar were painful stimuli always excluded.

The general effect of decreased alkaline reserve in hemorrhage and tissue-abuse in its relation to respiration is shown in figures 3 and 4. The experiments on hemorrhage are designated by an *H* and on tissue-abuse by *T. A.* and are shown in pairs for purposes of comparison.

Respiration and carbon dioxide content of the arterial blood are plotted on the ordinates against mean blood pressure on the abscissas. A definite similarity in the increased response of the center to decreasing alkaline reserve appears, but a better comparison is obtained by plotting the respiration against carbon dioxide capacity as shown in figure 5.

To control the results obtained in hemorrhage and tissue-abuse the effects of lowered alkaline reserve by acid injection (N/2 HCl) were first studied. Plotting the results in the manner described we find the reaction of the respiratory center to be represented by a straight line. (See fig. 5). Apparently this may be looked upon as the normal response.

Reduction of the alkaline reserve by a decreased volume-flow produced by hemorrhage elicits the same reaction. It would seem that the decreased volume flow per se accompanying the decreased alkaline reserve is without effect upon the respiratory center. But as pointed out before, the curve of flow through the submaxillary gland may not represent the flow obtaining under similar conditions through the brain.

The rapid rise of the curves of respiration in experiments 98 and 91 on tissue abuse represent exceptions to the previous reactions noted and suggest a reflex excitation of the respiratory center by painful stimuli. The disturbance in oxidations could hardly be marked

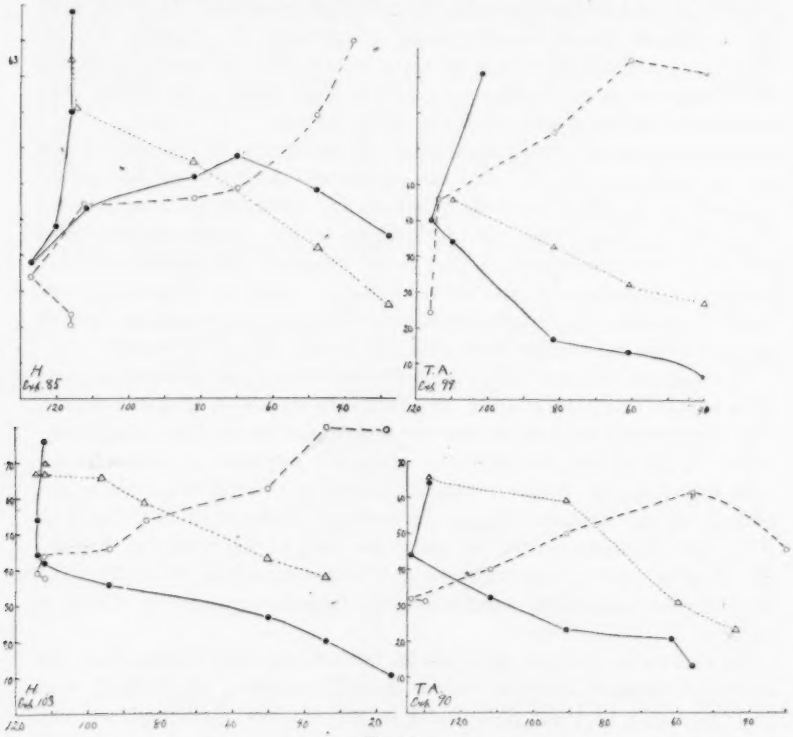

Fig. 3

Figs. 3 and 4. Comparison of conditions in experiments on hemorrhage, *H*, and tissue abuse, *T.A.* Basal flow, ● respiration, O, carbon dioxide content of arterial blood, △ and hemoglobin percentage plotted on the ordinates against mean blood pressure on the abscissas □. The figures on the ordinates represent carbon dioxide content; on the abscissas mean blood pressure in mm. Hg.

enough to account for the sudden diminution in alkaline reserve. See also experiment 98 as plotted in figure 4.

In experiments, however, where pain stimuli for some reason are less effective, the response of the respiratory center appears to be nor-

mal (see exps. 101 and 90 in fig. 5). The earlier failure of the center noted in certain cases of shock may represent a reaction to decreased body temperature from visceral exposure, a factor not carefully controlled.

If this is true cold is important from many points of view, not only in reducing blood-flow and oxidation, (35), (36), but depressing the

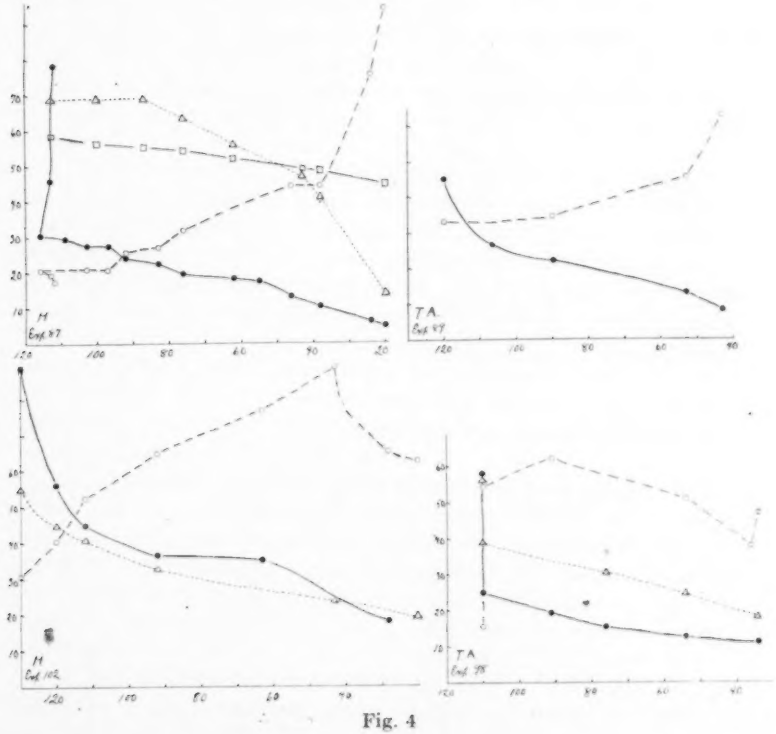

Fig. 4

respiratory center as well and from these experiments we infer that where the factor of cold does not enter the danger of hemorrhage and tissue-abuse is not attributable to a failure of the respiratory center. It may be, however, that the more shallow and rapid respirations in tissue-abuse are not as effective in securing gaseous exchange as the slower and deeper respirations in hemorrhage. No effort was made to determine the reason for the difference in the type of respiration. Pos-

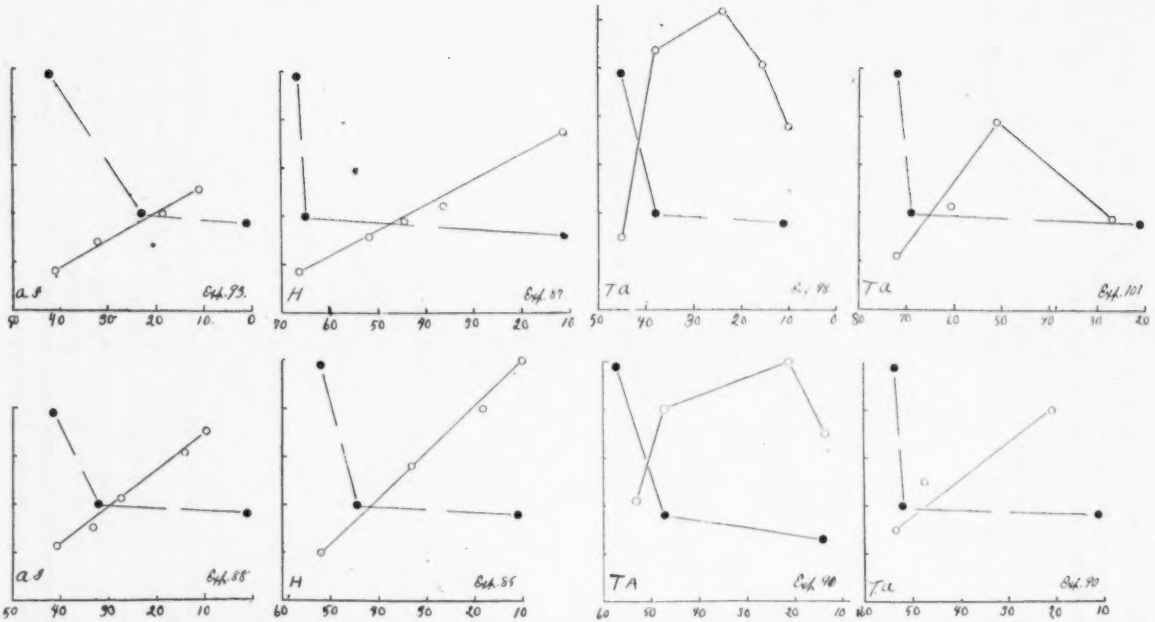

Fig. 5. Curves showing the response of the respiratory center to decreasing alkaline reserve in experiments on effects of acid injection, *A. I.*, hemorrhage *H* and tissue-abuse, *T. A.* Respiratory exchange is plotted on the ordinates against carbon dioxide capacity of arterial blood on the abscissas. Basal-flow of blood is roughly plotted on the ordinates.

sibly the opening of the abdomen interferes with the action of the abdominal respiratory muscles.

g. Comparative effects of gum acacia injection on the basal-flow of blood after hemorrhage and tissue-abuse. The experiments to be described were performed for a number of purposes:

1. To check the effects of inflammation on volume-flow of blood in the experiments on hemorrhage.

2. To determine the importance of the viscosity factor in volume-flow of blood in hemorrhage and in tissue-abuse.

3. To determine and compare in hemorrhage and tissue-abuse the manner in which volume-flow of blood responds to increased blood volume.

4. To arrive at some basis of treatment by taking into consideration the factor of volume-flow times the concentration of the blood.

g. 1. The similarity of the three curves of volume-flow in figure 6 obtained by bleeding, by acacia injection and by rebleeding indicate that the factors of cold and inflammation so far as they are concerned in the hemorrhage experiments have little influence on the volume-flow of blood. They show that severe hemorrhage if compensated in time is without serious effect upon the constrictor tonus and on the normal elastic properties of the vessels for the response is similar in three alterations of pressure. (See also curves obtained during bleeding and injection in fig. 7, exp. 49.)

g. 2. Changing the mean blood pressure by hemorrhage, by tissue-abuse and by acacia injection we get three combinations of varying pressures and concentrations of corpuscles. Assuming the corpuscle to be the main viscosity factor the curves of flow show that changes in viscosity occurring in these experiments have relatively little effect upon the configuration of the curve. The effects appear more upon its position.

g. 3. For example in experiment 49, figure 7, the injection curve differs from the hemorrhage curve mainly in position; this change is largely accounted for by dilution of the blood and by deterioration of the vasomotor center. The curve, however, represents a good response on the part of the vascular system to injections. The relatively insignificant effects of rise in pressure up to 85 mm. Hg. on the volume-flow and the enormous effects of a further increased pressure point to the practical importance of adequate injections in the treatment of lowered blood pressure. The shifting of the injection curve to the right indicates, however, that we should be satisfied in attaining pressures slightly below the normal pressure of the individual in question.

A prolonged condition of lowered blood pressure disturbs the normal response to injection. Apparently the vasomotor center and the elastic properties of the vessels are affected. The contour of the injection curve approaches a straight line. (See injection and rebleeding curve of exp. 48 and the rebleeding curve of exp. 49, fig. 7). This variation in response appears to be a matter of some importance relative to treatment of conditions of lowered blood pressure and will be considered below.

The experiments on the comparison of the response of the vascular system after tissue-abuse with the response in hemorrhage are limited. Figure 7 however shows that considerable similarity in the two conditions may be expected.

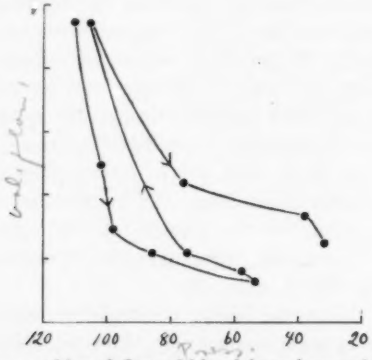

Fig. 6. Three curves of basal-flow of blood from hemorrhage, acacia injection and rebleeding.

Nevertheless, in certain instances (exp. 51, fig. 7) a shifting of the injection curve to the left occurred, an observation missing in the hemorrhage experiments, attributable I believe to the effects of a small fall in body temperature absent during hemorrhage.

g. 4. In the treatment of hemorrhage and shock transfusion both of blood and of inert solutions is practiced. So far as I am aware, apart from supplying fluid and fluid transport to the tissues, we have relatively little experimental basis for the treatment of conditions of lowered blood pressure by inert injection[3] and particularly hemorrhage

[3] By inert injection is meant the injection of a fluid so far as the tissues are concerned, physically and chemically inert, possessing the same colloidal and saline osmotic pressure and a similar viscosity as that of blood and having no chemically stimulating effect. A 7 per cent gum acacia suspension in 0.9 per cent NaCl may be looked upon as an approximation to such a solution.

and shock plus hemorrhage. The data we have is mainly of an empirical nature, namely, the beneficial effects following injections of saline solutions and of colloidal suspensions. It seemed, therefore, that if a basis for such treatment is found the procedures and details of administering injection might be improved. We would in addition have a basis for modifying the nature of the injection fluid to meet more nearly the needs of the tissues.

Looking upon blood as a means of transport of nutrient material to, and of waste material and secretions from the cells, we may be justified in assuming that either a dilution of the blood or a reduction in the flow would interfere with both of these functions. It is probable that the availability of any nutrient to the tissues depends upon the combination of two factors—volume-flow times concentration of the nutrient in question particularly when deficiencies exist. This combination I have called the *nutrient-flow*.

The damage done by a decrease in the nutrient-flow probably depends upon the extent to which any essential nutrient (oxygen, salts, alkali, derivatives of fats, carbohydrates and proteins) is depleted as the blood flows through the tissues. An important question, therefore, is to determine the particular deficiencies leading to disturbed nutrition.

It appears that the oxygen supply to the tissues is of utmost importance. This is more recently borne out by the demonstration of the equal effectiveness of the injection of red cell suspensions and of whole blood (37) yet it might be mentioned that the cells in this case were suspended in glucose solution. The beneficial effect and advantages of injection of sugar solutions noted by Sansum and Woodyatt (38), Erlanger and Woodyatt (39), Erlanger and Gasser and others (40), (41), (42), (43) and (44), in shock and in other conditions indicate the desirability of increasing the sugar concentrations of the blood. The unsettled views on alkaline reserve call for further study of this factor. This phase of the subject will be considered in greater detail in the discussion.

It is apparent that the increased volume-flow produced by transfusion of whole blood within certain limits might be of value in direct proportion to the increased flow. The question arises—wherein lie the beneficial effects of inert injection? Can the nutrient flow of any constituent be increased without its addition to the circulating blood? If so, the flow of blood must increase out of proportion to the extent of dilution eliciting the increased flow. That this occurs follows from the previous observation that a decrease in blood volume produces a

decrease in volume-flow out of all proportion to the decreased volume, and from the observation that the volume-flow curve with decreasing pressure may be duplicated with rising pressure from acacia injection.

The question, however, can be put to the direct test by measuring the volume-flow with decreasing and increasing pressure, multiplying

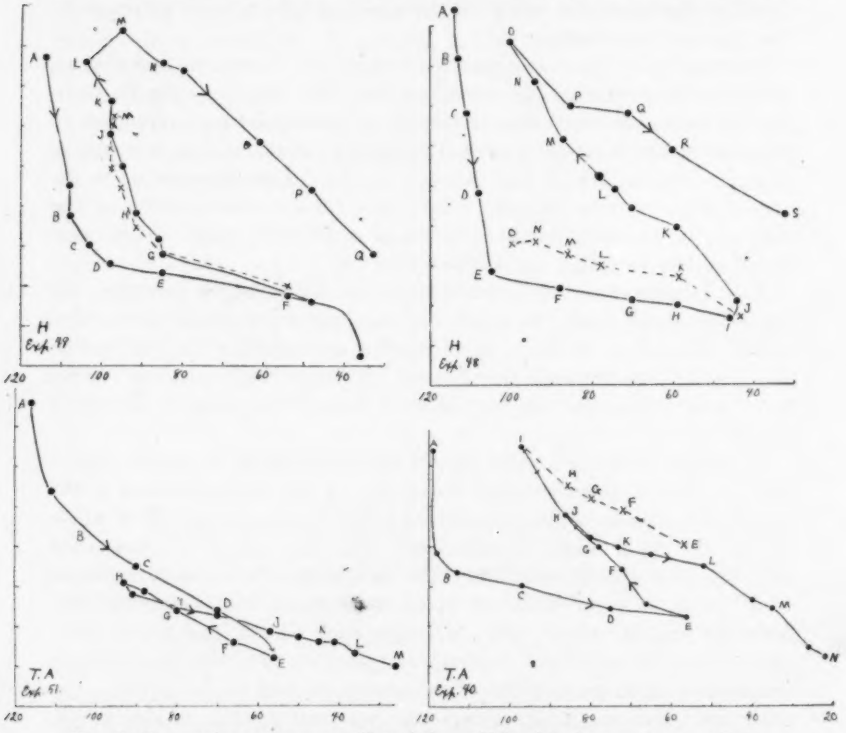

Fig. 7. Comparison of curves of basal-flow of blood in hemorrhage and tissue abuse showing the response of volume-flow to injection of acacia. With table 3 it illustrates the changes in nutrient-flow accompanying changes in volume-flow; the curve of nutrient-flow is represented by crosses. The curve nutrient-flow in experiment 51 follows the injection curve of flow.

by the percentage of red cells and obtaining the nutrient-flow. The data from four such experiments, two on hemorrhage and two on tissue-abuse, are shown in figure 7 and table 3. The data of the lettered observations are given in the table—blood pressure, volume-flow, percentage of red corpuscles or nutrient-concentration, nutrient-flow,

TABLE 3

Hemorrhage

EXPERIMENT 48							EXPERIMENT 49						
Observation	Blood pressure	Basal-flow	Percentage of red cells or nutrient concentration	Nutrient-flow	Nutrient-flow relative to initial nutrient-flow	Nutrient-flow relative to lowest nutrient-flow	Observation	Blood pressure	Basal-flow	Percentage of red cells or nutrient-concentration	Nutrient-flow	Nutrient-flow relative to initial nutrient-flow	Nutrient-flow relative to lowest nutrient-flow
A	114	94.0	55.6	5195	100.0		A	114	77.0	52.2	4020	100.0	
B	113	82.0	54.6	4476	86.3		B	108	37.0	51.8	1916	47.6	
C	111	68.0	52.9	3592	69.2		C	103	30.0	50.2	1506	37.5	
D	108	48.2	51.8	2497	48.3		D	98	25.6	50.0	1412	35.1	
E	105	28.7	50.5	1449	27.9		E	85	23.4	48.7	1139	28.3	
F	88	24.2	49.3	1193	22.9		F	84	17.5	47.0	822	20.4	100.0
G	70	21.3	49.0	1043	20.1		G	85	28.0	40.2	1124	27.9	142.5
H	59	20.0	48.8	976	18.8		H	91	38.0	37.3	1417	35.2	179.5
I	46	16.5	48.4	798	15.3	100.0	I	95	50.0	35.8	1791	44.5	227.0
J	44	21.0	41.7	875	16.9	112.7	J	98	58.0	34.4	1991	49.5	252.3
K	59	39.5	36.2	1430	27.6	184.1	K	98	66.0	32.9	2171	54.0	275.3
L	74	49.0	32.0	1568	30.2	201.2	L	104	76.0	32.0	2431	60.5	309.0
M	87	61.5	28.1	1727	33.3	222.0	M	95	84.0	30.0	2520	62.7	320.0
N	94	76.0	24.9	1893	36.5	240.0	N	85	76.0	28.5	2165	53.9	275.0
O	100	86.0	21.9	1884	35.5	236.7	O	61	56.0	24.2	1355	33.7	172.0
P	85	70.0	21.4	1498	28.9	192.7	P	48	54.0	23.4	1029	25.6	130.7
Q	71	68.0	20.9	1421	27.4	182.7	Q	33	28.0	21.7	604	15.0	76.5
R	60	59.0	20.3	1197	23.0	153.5							
S	32	43.0	16.2	688	13.2	86.7							

Tissue-abuse

EXPERIMENT 40							EXPERIMENT 51						
A	119	64.0	60.0	3840	100.0		A	116	76.0	65.6	4985	100.0	
B	118	38.5	67.0	2578	67.2		B	100	46.0	70.4	3235	65.0	
C	97	30.5	70.0	2134	55.6		C	90	35.0	70.6	2471	49.6	
D	75	24.5	73.0	1788	46.6		D	70	24.0	73.2	1757	35.2	
E	56	22.0	72.0	1584	41.2	100.0	E	56	12.0	69.4	832	16.7	100.0
F	72	34.5	54.4	1875	48.8	118.5	F	66	16.0	60.6	969	19.4	116.0
G	78	40.0	49.8	1992	51.9	125.7	G	80	23.0	55.3	1265	25.4	152.0
H	78	48.0	44.0	2112	55.0	133.5	H	92	31.0	48.6	1504	30.2	181.0
I	97	65.0	37.9	2462	64.2	156.0	I	80	25.0	44.4	1110	22.3	133.5
J	85	47.0	37.0	1740	45.3	110.0	J	57	18.7	43.4	811	16.3	97.6
K	73	40.0	36.4	1455	37.9	92.0	K	45	16.0	42.4	675	13.5	80.8
L	52	33.0	35.7	1178	30.7	74.5	L	36	13.0	41.3	537	10.8	64.6
M	35	24.0	34.1	816	21.2	51.4	M	26	9.8	42.8	419	8.4	50.2
N	22	12.0	33.1	396	10.3	25.0							

nutrient-flow compared with the normal initial nutrient-flow valued at 100, and the nutrient-flow relative to the lowest nutrient-flow also valued at 100. The curves of nutrient-flow as well as of volume-flow are shown in figure 7.

In experiment 49 on hemorrhage the pressure was lowered to 36 mm. Hg. The nutrient-flow was decreased to less than 20 and was raised again by acacia injection to 62.7. The value of the highest nutrient flow at *M* compared with that at *F* is 309.

In experiment 48, also on hemorrhage, the nutrient-flow was decreased to 15.3 but subsequently raised only to 36.5.

The two experiments on tissue-abuse also represent what might be looked upon as a successful and less successful treatment. In one case the nutrient-flow was lowered to 41.2 and raised to 64.2, in the other lowered to 19.4 and raised to 30.2. The failure to improve the nutrient-flow more in these experiments is largely due to a failure to inject enough acacia. For example, in experiment 51 the percentage of corpuscles at the height of injection was 48.6. Further dilution undoubtedly would have been accompanied by an increased nutrient flow.

It is apparent from these tables that in both hemorrhage and in tissue-abuse the condition of lowered blood pressure may be markedly improved by the injection of an inert solution.

In attempting to explain the increased nutrient-flow accompanying increased volume-flow from inert injection the factor of pressure is practically eliminated by analysis of conditions represented in figure 7 experiment 49, where pressure remains constant but volume-flow rapidly increases. By assuming the function of the heart to remain constant the dependence of volume-flow is reduced to three main factors—viscosity, caliber of the vessels, and filling of the heart. All of these factors are undoubtedly operative in increasing the flow.

Concerning viscosity, Tigerstedt noted that relatively large injections of defibrinated blood affected little the ventricular output of the rabbit while similar injections of salt solution augmented the flow. The difference in results he attributed to the diminution of viscosity of the blood by saline injection. This effect of decreased viscosity is also noted in the shifting of the injection curve of volume-flow to the right in figure 7 of this paper. But if this factor is operative in increasing the nutrient-flow it must decrease the viscosity out of proportion to the dilution entailed. An analysis of the tables of Burton-Opitz would indicate this as a possibility if the results of viscosity as measured by

flow of blood through a glass capillary tube can be applied to the peripheral flow of blood in situ. Though a change in viscosity may influence the nutrient as well as the volume-flow it does not appear to be a factor when gum acacia is injected, as is indicated by the relatively small effect of change in viscosity (as measured by the corpuscles) on the curves of volume flow. This is further borne out by the relatively small increase in nutrient and volume-flow elicited by acacia injection in experiment 48 when compared with experiment 49.

Some other factor comes into play. In later experiments controlling the effects of small intravenous injections of drugs on cardiac output, Tigerstedt noted that an increase of the normal blood volume augmented the cardiac output out of proportion to the injection. The curves of volume-flow of blood of the submaxillary gland show that in all stages of depleted blood-volume injection of inert solution may increase the volume-flow out of all proportion to the dilution produced. We know from the increased filling of the heart which occurs that dilution results both from the increased capacity of the heart and of the larger vessels, and from the increased volume-flow occurring with constant head of pressure that dilution must also result from the increased capacity of the peripheral vascular system. It is of interest, therefore, to know how the nutrient-flow increases despite these two dilutions.

The explanation of this phenomenon, I believe, is to be found in the application of Poiseuille's law for the flow of fluids through tubes, in conjunction with changes in the capacity of the vascular system accompanying increased blood flow. By experiments upon the flow of distilled water in capillary tubes 0.15 to 0.65 mm. in diameter Poiseuille reached the conclusions represented in the formula $A = \frac{kR^4}{L} H$. A, volume outflow per second, R radius and L, length of the tube, H, the pressure head, and k the transpiration coefficient.

Assuming the length of the peripheral vessels directly determining the volume-flow to remain constant the conclusion in which we are particularly interested is that with the same head of pressure the time spent in the outflow of a certain volume of fluid through equally long tubes is inversely proportional to the fourth power of the diameter of the tube. Assuming further that the capacity of the vascular system increases only through increased caliber of the peripheral vessels it must follow that in the increased volume-flow and the increased caliber of the vessels occurring in the sharp ascent of the curve of basal-flow during injection, that the volume-flow increases directly as the fourth

power of the caliber of the vessels while the volume of the vessels or the dilution increases only as the second power of the caliber.

A careful application of this relation has not been made but it is obvious that in the relation of the fourth to the second power great possibilities for improvement of nutrient-flow by simple dilution exists. For working out these possibilities, however, we need information on the diluting effects of increased capacity of the larger vessels produced by injection. The relative significance in changes in the arteriole and capillary bed must also be considered.

It will be noted that the nutrient-flow increases during the early stages of injection while the peripheral vessels are contracting as well as in the later periods where dilatation occurs. The increased nutrient-flow may be explained by bearing in mind the effects of increasing head of pressure and the observation of Pilcher and Sollmann that at the lower pressures small volume changes are most effective in producing pressure changes.

Though the experiments described represent only a preliminary study on nutrient-flow they suggest some points of significance in treatment.

Note that the experiments on hemorrhage and tissue abuse in which nutrient-flow was most improved are those in which the injection curve of flow more nearly approximates the first curve. In these instances the decreased flow preceding injection has had relatively little effect upon the vasomotor center and on the elastic properties of the blood vessels. Note also that little effect on nutrient-flow is obtained until a relatively high pressure is reached when a sudden increase occurs; emphasizing the importance of not only attaining a high pressure but of increasing the blood volume still more to overcome the vasoconstriction.

If the decreased flow is prolonged and has resulted in greater changes in the vessels and in the vasomotor center as suggested in experiment 48, the relation of volume-flow to increased blood volume is much less favorable for successful treatment. The normal response of volume-flow to injection is missing, the amount of dilution approaches more nearly the increased volume-flow of blood and the result is a relatively smaller improvement in the nutrient-flow.

The more rapid increase in volume-flow with an elevation of pressure at the lower levels indicates a failure of the peripheral vessels to contract and may represent a general failure of the vascular system which requires for a given increase in volume-flow a greater dilution of the circulating blood, thereby counteracting the beneficial effects of

dilatation previously outlined. We are reminded here of the failure of the veno-pressor mechanism, (45), (46), but here, too, the relative effects of changes in the arterioles and capillaries must be taken into account.

Note that in the better reactions the curve of nutrient-flow parallels more closely the curve of volume-flow.

Between observations *N* and *O* in experiment 48, the mean blood pressure increased 6 mm. Hg., the volume-flow increased from 76 to 86; the nutrient-flow, however, decreased from 36.5 to 35.5. With further injection blood pressure measurements undoubtedly would have pointed to an improved circulation though damage was possibly occurring. The observations point to a limitation of the beneficial effects of acacia injection.

The condition of lowered blood pressure is a critical one and calls for radical treatment. The blood volume should be increased by transfusion of blood or blood substitutes. Gum acacia theoretically should and practically does give beneficial effects. As Bayliss (47) showed, it has many of the desirable properties of blood and is inert and "perfectly innocuous." Even after large injections of concentrated suspensions Meek and Gasser (48) and Gasser, Meek and Erlanger (49) find no harmful effects in normal animals. If that is true methods should be available for pushing the treatment to the limit of its beneficial effects especially when blood is not available.

It should be borne in mind in pushing the injection that in addition to the over-dilution there is the danger of transudation augmented by elevated pressures. Herein lies the great difficulty of treatment in serious cases of shock and of hemorrhage going into shock, where transudation is free.

DISCUSSION

Nature of shock. The preceding data show the reactions common to hemorrhage and tissue-abuse,—the initial rise of pressure, the marked vasoconstriction, the relation of volume-flow of blood to blood-pressure, the disproportionate decrease in volume-flow to decrease in blood volume, the decrease in alkaline reserve, the normal response of the vasomotor and respiratory centers and the response of volume-flow to rise of pressure with acacia injection. In addition we may have dehydration of certain tissues, the symptoms of thirst, an increased peripheral red count, etc., in both hemorrhage and shock. (The similarity of the two conditions in connection with the increased sugar

and adrenin content of the blood has been more recently brought into question by Stewart).

There are, however, some conspicuous points of difference. In hemorrhage the accessory initiating factors doing tissue damage and producing additional nutritional disturbances may be largely wanting. The condition is primarily a general disturbance resulting from a decreased blood volume and volume-flow accompanied by a dilution of blood which is a response of normal tissue. The condition of shock, however, may begin as a local or general disturbance permitting concentration of the blood from transudation and resulting in a decreased blood volume and volume-flow. In hemorrhage the ability to recover lost blood volume is little affected. In the early stages of shock this may likewise be the case as was shown by Cobbett and Roy (50) and by Vale (51). Though the intestines may increase in volume from transudation during exposure the percentage of corpuscles remains constant. The loss of plasma to the intestines shown by the decreased specific gravity of the intestines is made good by the fluids from other tissues as is indicated by the increased specific gravity of the muscle. The accessions of fluid from the more normal tissues, however, soon fail to keep pace with the fluid lost and concentration occurs. Concentration of the blood in accordance with these findings represents a local rather than a general condition and may over-emphasize the difference between hemorrhage and shock; yet Vale who confirms the results of Cobbett occasionally finds that the decreased specific gravity may occur under special circumstances in all the tissues. Gasser and Erlanger conclude from a study of blood colloids that in aortic shock dilution of the blood may occur in some parts of the body while concentration from transudation occurs in other parts. It would seem that the extent to which concentration is due to a local or general condition varies considerably and may be a matter of significance both concerning the nature and treatment of shock. One is reminded again of experiment 100 in which the local inflicted injury was insufficient to account for concentration from local transudation.

The greater severity of shock may be attributed first to the fact that shock is a result of tissue damage while in hemorrhage tissue damage may occur only from prolonged anemia. In shock the deteriorated tissue withstands more poorly a reduction in volume-flow than does the more normal tissue in hemorrhage. In hemorrhage the volume-flow of blood is improved by dilution of the blood; in shock the organism, on account of increased permeability, cannot take ad-

vantage of the favorable relation of blood volume to volume-flow. While in hemorrhage a limit is set to the transfer of fluids from the tissues to the blood by the beneficial effects derived therefrom, no limit is set in shock and the drain and transfer of fluid continues until the mechanism breaks down.

It may be that in prolonged hemorrhage, however, these points of difference disappear, the tissues suffer damage, the maintenance of blood volume is affected and the two conditions merge. In that case, disregarding other effects of tissue-abuse, the difference between hemorrhage and shock is one more of sequence of processes than of nature of processes.

There is considerable difficulty in bringing into proper relation the effects of injury and hemorrhage in conditions of lowered blood pressure, particularly at the front where the two seldom occur alone, but together in varying proportions. Surgeons recognize hemorrhage to be the most serious shock-producing factor; yet attempts are made to determine the relative importance of shock and of hemorrhage. One finds this statement for example, "It seems reasonable to conclude that the cause of death, while in part hemorrhage, was largely shock. The total amount of blood lost did not in itself appear to be sufficient to cause death." While a partial differentiation may be made in some cases yet if the factor of disturbed circulation is of importance, difficulties will be met for it may be a matter of indifference to the tissues in general whether the volume-flow is diminished as a result of external hemorrhage or of internal hemorrhage and transudation. Recall, e.g., experiment 100 cited on page 478.

The relative dangers of hemorrhage or any other form of tissue-abuse vary with the condition of the individual. Looking at shock in its broadest way from the observations of Fraser and Cowell (52) it would seem that shock begins when the individual moves from the resting quarters to the fighting area where the strain is intense. This is borne out by the findings of Robertson (53) on the effects of bleeding on the donor. "Even a slightly wounded man straight from the trenches withstands the loss of blood poorly compared with a man (convalescing in the rear) with a sprained ankle due, I believe, to the shock of battle."

When we bear in mind the concentration of the blood and the deteriorated condition of the tissues resulting from exertion, the inflicted injury, hemorrhage of varying degrees, exposure, etc., the prevalence of shock at the front is comprehensible. The more frequent occur-

rence of shock in the routed than in the victorious army is interesting in this connection.

Though shock is looked upon in this general way it may appear that the factors of decreased blood volume and volume-flow of blood have been over-emphasized. It is realized that various forms of tissue-abuse cannot help but have disturbing effects upon tissue nutrition for without these effects an increased permeability, transudation and decreased volume-flow could never result. And it might be stated here that the observations on the importance of pain as a shock-producing factor, the central influence over processes in the periphery, the liberation of toxins in the injured part, etc., have not been disregarded. In limiting the work to volume and nutrient-flow I do not feel that the point of view of shock has been narrowed for all the normal processes of nutrition are dependent upon an adequate volume-flow of blood. That various forms of tissue-abuse and tissue deterioration are best combatted by a free circulation appears well supported by the universal recognition by surgeons of the danger of hemorrhage.

Shock is, therefore, looked upon as a general deterioration of the tissues resulting from tissue-abuse. The misleading nature of the term shock as originally used is well recognized and is due to the emphasis on a disturbance of a single system in the organism (54). The picture of secondary shock, however, is a general one of disturbed circulation and nutrition arising from many causes more limited and local in some cases than in others. Since in every case the initial disturbance is tissue-abuse of some form leading to a general slowing of the circulation, the term *traumatic-hypodromia* appears general enough to meet all conditions and specific enough to be of some descriptive value. Since shock cannot be defined by blood pressure levels it has the advantage of applying to all stages of shock whether the pressure be normal, above or below normal.

Treatment. It is probable that the processes set up by manipulation of the intestines, by crushing of muscle, extensive burns and interference with the blood supply, etc., will present differences and yet certain essentials such as decrease in blood volume with its effects on volume-flow will be common to all. There will then be at least one common treatment, the proper increase of blood volume.

By a proper increase in blood-volume is meant one which will *a*, combat increased permeability; *b*, insure a supply of fluid to the dehydrated tissues; *c*, elicit an increased volume-flow to overcome the stasis and *d*, improve the nutrient-flow to overcome the nutritional disturbance.

a. The permeability may be combatted indirectly by improved nutrition of the tissues and directly by proper attention to the physical and chemical properties of the injected fluid. This is a problem in itself and was not made a point of special study.

b. In many cases of shock, particularly where there is a marked local transudation, dehydration of tissues may be even more pronounced than in hemorrhage. In hemorrhage all the fluid must be supplied from the exterior. In shock without hemorrhage a redistribution of the fluid by the intravenous injection of hypertonic solutions producing *internal transfusion* is a possibility. The other method consists in supplying fluid which will saturate all the tissues leaving the locally damaged tissue over-hydrated. The choice of method of increasing blood volume and supplying tissue fluid would seem to depend on the relative dangers of dehydration and over-hydration, on the relative importance of local and general transudation (55), (56), (57), (58).

c and d. In this research only the effects of external transfusion are dealt with in their relation to volume- and nutrient-flow. It might be noted that insofar as internal transfusion produces a dilution of the red cells and of the blood colloids the effects which it produces on the nutrient flow as regards some constituents, at least, are comparable to the effects of inert injection. But the suggestion of Erlanger and Gasser that hypertonic injection attracts a well balanced salt solution into the circulation should be noted.

The results on nutrient- and volume-flow obtained in this research were discussed before. Here we are interested in their application. The nutrient-flow is probably most improved by transfusion of whole blood, but it may likewise be increased several hundred per cent by injection of inert solution. The question, therefore, arises which of the methods of external transfusion should be used to combat conditions of lowered blood pressure.

The answer to this question depends largely upon the severity of the case, upon whether a nutrient-flow below normal will suffice, and on the relative importance of various nutrients and their minimum concentration compatible with normal nutrition.

The disturbances of oxidation noted point to the prime importance of oxygen, yet a lack of other nutrients might also lead to acid products of incomplete oxidation. The experiments of Abel, Rowntree and Turner (59) in which repeated hemorrhage was produced, returning only the corpuscles suspended in Locke's solution, show that relatively

little plasma is essential to life. The equally good results of transfusing red cells and whole blood in man also point to the red cells as the important factor (60). Rous and Wilson (61), on the other hand, substituting horse plasma for whole blood in the rabbit, show the very small amount of hemoglobin absolutely essential to life. Mann (62) obtains best results in the treatment of shock with the transfusion of serum.

In rabbits reductions of hemoglobin to 20 per cent are survived. Much lower figures in man are on record. At the front experience calls for transfusion of blood when the hemoglobin has fallen to 20 or 30 per cent, 30 or 40 per cent is still grave but percentages above 40 per cent are no longer serious (63), agreeing with the results of Bayliss (64) who finds a loss of blood of 50 per cent to be combatted with acacia, more serious hemorrhages requiring transfusion of blood

At the Fourth Inter-Allied Surgical Conference, March 11 to 16, 1918, transfusion of blood was recommended as the method of choice in the treatment of hemorrhage. In view of the improvement and the safety of transfusion of blood and the fact that whole blood increases the nutrient-flow to a maximum, critical cases at least call for such treatment but it seems that injection of acacia has a definite place in the treatment of less serious conditions. Such injections undoubtedly would be of great benefit in cases with high pressure and moderate hemorrhage,—hemorrhage, however, which along with other forms of tissue-abuse, is sufficient to initiate the condition of shock. Administration of such preventive or rather early treatment requires the early recognition of circulatory disturbances. If acacia treatment is pushed to the limit in serious cases where blood in unavailable special precautions would also be required to avoid over-injection.

As regards shock the Inter-Allied Conference felt that indications for transfusion of blood were not as well defined as in hemorrhage. In hemorrhage, if the tissues are sound, tissue damage and loss of maintenance of blood volume result only after prolonged decreased flow and therefore if the condition is combatted early good results are to be expected. In shock the processes may be reversed, the decreased flow coming on as a result of tissue damage. The dangers are less apparent and probably more serious. Though robust individuals may be more susceptible to the development of shock, (Cobbett and Erlanger and Gasser) the severity of many cases is probably due to the reduction of the factor of safety to the minimum when the disturbance is treated. That the factor of safety is variable even in apparently

healthy individuals is indicated in figures 3 and 4. Note for example the relatively great resistance displayed by dogs 87 and 103. Though the volume-flow of blood is markedly reduced the oxidations as indicated by the horizontal curve of CO_2 content are little affected. When the factor of safety is consumed, however, the break is sudden and serious as indicated by the deflection of the respiratory and CO_2 gradients. The slight resistance of dogs 85 and 102 is indicated by the prompt deflection of the gradients at the outset of the experiments.

The earlier stages and the less serious cases of shock should show a more uniform response and might well be treated with various forms of gum acacia injection so as to restore the original volume of blood. If no hemorrhage occurred, but rather concentration of the blood, the corpuscular element, provided stasis is not marked, might not suffer dilution below the normal. This may also hold to some extent for the blood colloids for Gasser and Erlanger, and Lazarus-Barlow found the concentration of these colloids to increase with transudation. Therefore, after removing the initiating factors of shock the increased volume- and nutrient-flow may in themselves lead to recovery by improving nutrition and checking further transudation directly and indirectly.

But shock with hemorrhage and serious cases of shock in which the capacity of the vascular system has increased might respond better with blood transfusion or at least a mixture of blood and acacia suspension. The dilution of blood from acacia injections requisite to produce the normal volume-flow would probably lower the nutrient-flow below that compatible with recovery. Whether the failures of treatment of shock by transfusion of whole blood are due to transfusions inadequate to maintain increased volume-flow for a long enough period, or to the irreversibility of vital processes should be determined by the employment of larger transfusions.

The suggestions derived from this work in regard to treatment are *a*, The importance of recognizing the relative dangers of various forms of tissue-abuse,—the initiating factors; *b*, The importance of recognizing the condition of shock in its earliest stages before the mean blood pressure has fallen and serious damage has been done; *c*, The need of developing methods of transfusion insuring the maximum nutrient-flow compatible with the conditions prevailing; *d*, The desirability of developing special methods, other than improved nutrient flow, to combat the permeability of the peripheral vessels.

(Among the initiating factors should be included the factors which come into play before as well as during and after the operation, for ex-

ample discomfort, hunger, thirst and dehydration of the tissues from purge entailed in the not uncommon preparation of the individual for operation. The dangers of preoperative dehydration has been recognized by Cobbett, and Lazarus-Barlow has shown that saturation of the tissues with water tends to prevent concentration of the blood. The enormous effects of a small concentration of blood upon the volume-flow shown in this paper indicates the advisability of preoperative hydration rather than dehydration of the tissues.)

SUMMARY AND CONCLUSIONS

The object of this and the preceding research is to learn more nearly the nature of shock, to determine how closely normal cell nutrition is dependent upon normal volume-flow of blood and to study the factors controlling volume-flow so that this may be more exactly controlled in the treatment of conditions of lowered blood pressure.

On account of the similarities of hemorrhage and shock, the frequent synchronous occurrence of these conditions and the recognition of hemorrhage as "the most potent shock producing factor," this object seemed best attainable by a comparative study of the two conditions.

With a variety of methods employed a close dependence of normal nutrition upon normal flow of blood was indicated.

The curves of basal-flow of blood plotted against mean blood pressure are similar in hemorrhage and tissue-abuse. They show:

a. That a reduction of volume-flow amounting to 85 per cent of the initial flow may occur with a constant head of pressure or with a small change in head of pressure—a rise as well as a fall.

b. That this sudden decrease in volume-flow may after a time be as suddenly checked; with a further fall in pressure the flow may for a time remain constant, decrease or even increase.

c. That the initial decrease in volume-flow far exceeds that occurring during the subsequent fall in pressure to zero.

The similarity of the curves of basal-flow of blood in hemorrhage and tissue-abuse suggests similar vascular reactions of similar origin,—a change in caliber of the vessels being the most important factor underlying the deflection of the curves.

A decrease in blood volume of less than 10 per cent produced by hemorrhage may elicit through constriction of central origin a decreased flow of blood through the submaxillary gland of more than 60 per cent even though accompanied by a rise in pressure.

Tissue-abuse produced by manipulation of the intestines may produce a diminution in volume-flow equally as great, often accompanied by a rise in pressure.

Concentration of blood is sufficient to explain this vasoconstriction, but its effects are probably augmented by a shunting of blood into a directly abused area.

The dilatation following constriction in both hemorrhage and tissue-abuse appears attributable mainly to deterioration of the vasomotor center.

The superbasal-flow of blood in tissue-abuse as well as in hemorrhage decreases out of all proportion to the decrease in mean blood pressure.

This decrease is attributable to the increased constrictor tonus which must be inhibited and to the decreasing stretching and driving head of pressure which takes advantage of the inhibition.

The changes in basal- and super-basal flow along with the close dependence of normal nutrition on normal flow of blood explain the changes in alkaline reserve in hemorrhage and in tissue-abuse.

The relation of alkaline reserve to volume-flow of blood was considered.

The respiratory exchange in experiments on acid injection and on hemorrhage when plotted against carbon dioxide capacity was represented by a straight line. This presumably indicates a normal response of the respiratory center to decreasing alkaline reserve.

In tissue-abuse variations of this response occurred and are attributed at present not to an abnormal reaction of the center to chemical stimulation, but rather to a varying rôle played by pain reflexes.

The curves of basal-flow of blood in hemorrhage may be duplicated in the reverse direction by injection of gum acacia and then reduplicated by a second hemorrhage.

In tissue-abuse the response appears to be similar. After tissue damage occurs as a result of prolongation of the decreased volume-flow, the normal reaction of volume-flow to intravenous injection disappears.

By taking into account the factor of volume-flow times concentration of blood, as measured by the red blood cells, a basis for treatment by injection of inert solution may be worked out.

This factor designated as *nutrient-flow* may be increased several hundred per cent by inert injection.

This increase can be attributed only to the fact that the volume-flow is increased out of all proportion to the dilution of the blood eliciting the increased flow. It is in agreement with the observation that a

decrease in volume-flow is out of proportion to the decrease in blood volume eliciting the decreased flow. It is explained by an application of Poiseuille's law of flow of fluids through capillary tubes.

If the effects of decreased flow are prolonged the capacity of the vascular system increases to such an extent that dilution by injection now approximates more closely the increased volume-flow, thereby retarding the increase in nutrient-flow.

Despite an increased volume-flow and an increased mean blood pressure, elicited by injection a decrease in nutrient-flow may occur.

Such conditions seem to call for transfusion of blood or at least a mixture of blood and acacia.

The conditions developing in hemorrhage and shock are shown to be same in many essentials.

Hemorrhage if left untreated may merge into the picture of shock. There are, however, important differences of which sequence rather than nature of processes and the disturbed function of maintenance of normal blood volume appear the most significant.

Since the combatting of various forms of tissue abuse on the part of the animal depends to a large extent upon an adequate volume-flow, the effects of even a slight hemorrhage illustrate why it is recognized as the most serious occurrence for the development of shock.

Shock is looked upon in a very general way as a combined circulatory and nutritional disturbance mainly of peripheral origin. The condition is initiated by a variety of forms of tissue-abuse producing tissue damage, transudation of plasma and stasis of blood. The decreased blood volume eliciting a vascular constriction reduces the flow of blood to a level below that essential for normal nutrition and far below that essential for deteriorated tissues. The condition is thus sustained by the disturbed nutrition and by the consequent inability on the part of the animal to recover the normal permeability of the vessels and therefore restore the normal blood volume that is so essential for an adequate flow of blood.

Since the circulatory and nutritional disturbances are so closely interdependent, one disturbance may become as important as the other.

Of all the numerous vicious cycles the interdependence of volume-flow and blood volume with its consequent effects on nutrition and transudation appears the most serious.

Whether the disturbed function of maintenance of blood volume requires specific treatment or is amenable to treatment by improving the volume and nutrient-flow with the transfusion of blood or acacia as employed in this research was not studied.

If improvement of the nutrient-flow is an effective measure of combatting transudation it appears that similar principles of treatment with certain modifications can be applied to hemorrhage and to shock.

The suggestions on treatment of shock derived from this work are:

a. The necessity of recognizing more fully the major forms of tissue-abuse so that shock may be more frequently prevented.

b. The need of early detection and treatment of circulatory disturbances before serious damage has been done.

c. The need for the development of methods to guide in the amount and nature of transfusion.

d. The desirability of directly as well as indirectly combatting the increased permeability of the peripheral vascular system.

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STUDIES ON THE SUBMAXILLARY GLAND

V. An Automatically Filling and Recording Spirometer

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The disturbance of normal oxidations noted in conditions of lowered blood pressure may be ascribed in large part to the decreased volume-flow of blood, but the problem of oxidation is of such importance that it calls for study from various angles. By following the alkaline reserve we get an indication of the completeness of the oxidative processes and find that with decreasing volume-flow of blood there is a progressively increasing rate of neutralization of the alkaline reserve. As the alkaline reserve of the blood diminishes, two things may happen: an increased hydrogen ion concentration in the blood or an increased ventilation of the blood flowing through the lungs preventing this concentration.

It was to study in a quantitative way the reaction of the animal to decreased alkaline reserve accompanying decreased volume-flow of blood in hemorrhage and tissue abuse that this method of recording respiration was devised.

Though a large Hutchinson spirometer may be used to measure either expired or inspired air over relatively long periods of time and may frequently possess certain advantages, it has the disadvantage of lending itself poorly to the graphic method of recording and consequently is not well adapted to record irregularities and changes in respiration which may occur from time to time.

The automatically recording spirometer meets these disadvantages. Its operation is simple and it is, therefore, described (see fig.1).

The device is a modified Krogh spirometer, filled to a constant level with room air during each expiration. This air, then at the disposal of the animal, is partially inspired during each succeeding inspiration. The decrease in the volume of the spirometer occurring with each

inspiration is graphically recorded and by calibrating the instrument the amount of air inspired can be determined. The device operates automatically by means of a system of valves, an electrical contact and a motor.

In the spirometer used in the present research the float, *1*, has a capacity of 1000 cc. This float is pivoted at *2* and balanced by weight, *3*. It is in communication with the exterior by means of valve, *4*, which permits the passage of air only from the exterior to the interior. The spirometer also communicates with the animal through tube, *4'*, the ether bottle, *5* and the double respiratory valve, *6*.

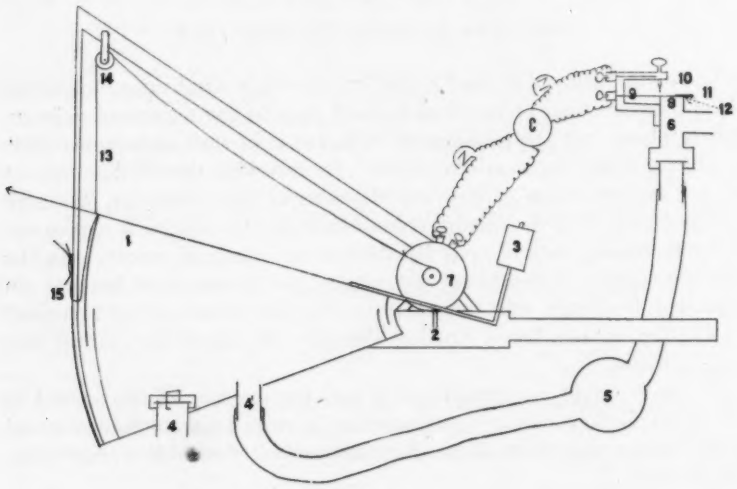

Fig. 1.

The double respiratory valve is the usual valve employed for separating expired from inspired air. It is so connected that inspired air is drawn from the spirometer and exhaled again to the exterior.

The upper or expiratory valve, *8*, made of insulating material (mica), is fitted with an electrical contact for activating the motor, *7*, which fills the spirometer with air. The valve is kept in place by means of a strip of tinsel, *9*, soldered to the platinum plate, which serves as an electrical connection. With each expiration the platinum plate is blown against the adjustable platinum contact, *10*, which closes the motor circuit and the spirometer fills.

Much depends upon the working of this valve. It is therefore described in some detail. In the ordinary respiratory valve the movements of the expiratory valve are very uncertain. If the respirations are large, the valve may be lifted bodily off its seat insuring a good contact. Very often, however, the amount of expired air is exceedingly small and the movements of the valve proportionately slight. Differences in adhesion are sufficient to make the valve open, now at one point and now at another. It was therefore desirable to devise a valve which would move freely and in the same way, with each expiration, giving a proper contact with the smaller expirations, and yet having sufficient capacity for the deepest respiration. One of the methods which has proved satisfactory is described.

The valve is weighted by a piece of lead foil, *11*, about 5 x 10 x 0.5 mm. fastened to the upper surface with Horsely's wax in the position shown. The placing of the lead foil prevents, momentarily, the rising of the valve as a whole. The lighter inner side rises to the contact, the valve turning on a hingeless joint made by placing the center of the weight over the edge, *12*.

The smallest expiration can be made effective in closing the motor circuit by adjusting contact, *10*. The opening of the valve on the contact side may with a fine adjustment be too small to allow the passage of the expired air when the respirations are increased, but when that occurs the platinum plate, blown up against the contact, forms a new pivot above and the outer edge of the valve becomes the freely moving part. It requires only a small additional increment of pressure to open this edge, the extent of this opening varying with the amplitude of respiration. The valve, therefore, adjusts itself automatically to changing magnitudes of respiration.

The operation of the apparatus requires little attention. When the electrical contact at *10* is made during expiration, the motor is activated and the float is drawn up by cord and pulley, *13* and *14*. During inspiration the motor unwinds with the descent of the float. A horizontal base line, easily adjusted to any height is obtained by checking, at a constant point, the ascent of the float, by means of a cord attached thereto.

During expiration when the float is raised the animal is protected by valve *6* against any negative pressure which may develop. Since this valve closes, room air only enters the spirometer through valve *4*. During inspiration valve *8* closes. The only air accessible to the animal is that contained in the spirometer,—this air passing

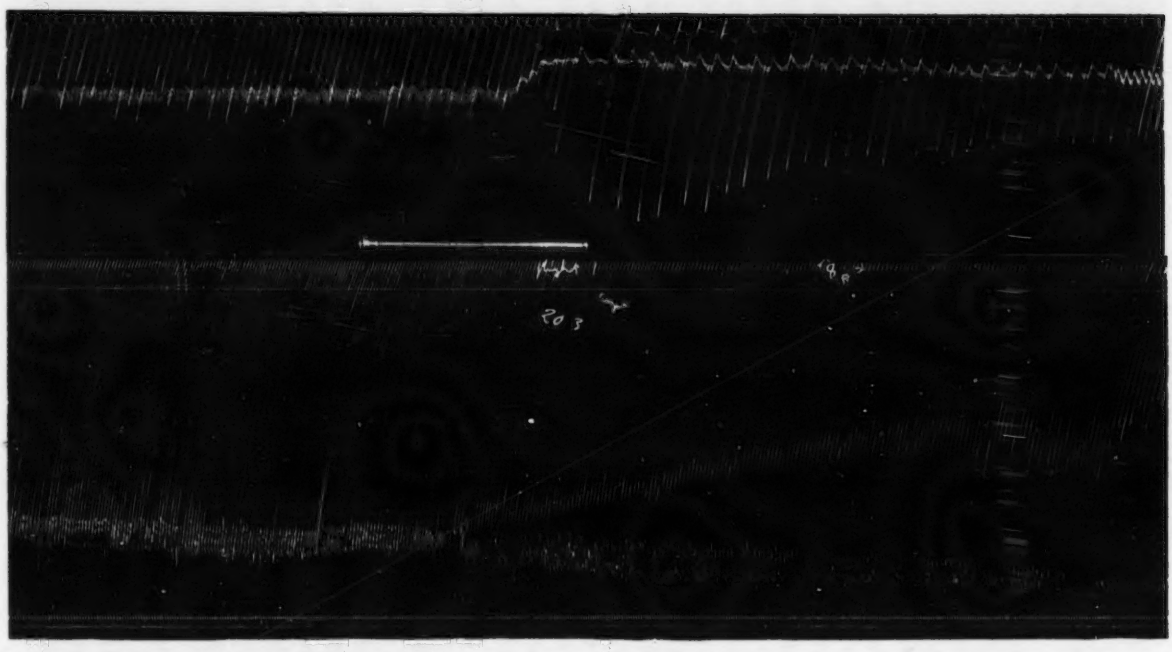

Fig. 2. Record showing changes in respiration. Upper tracing, effect of vago-section. Lower tracing—effect of lowered alkaline reserve. (Tracing taken immediately before death from acid injection.)

through valve *6*. Valves *6* and *8* are light valves the exact weight of which is of little importance for the operation of the spirometer. The weight of valve *4*, however, is of considerable importance. It must be heavy enough so that during a strong inspiration the float is drawn downward without the entrance of air through valve *4* and it must be light enough and large enough not to offer undue resistance to the entrance of air when the float is raised. The disadvantages of much resistance to the entrance of air are evident. It requires the employment of strong forces, the consumption of considerable electrical energy with the production of fling and vibration. By grading the area and thickness of the aluminum valve, *4*, and employing a minimum current in the motor these difficulties are met.

A record of respiration obtained with this device on the dog is shown in figure 2.

THE EFFECT OF OSMOTIC PRESSURE ON THE EXCITABILITY OF THE NERVE

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The sixth contribution to the physiology of osmotic pressure, from the Physiological Institute, Imperial University of Kyoto, Japan

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INTRODUCTION

The effect of the osmotic pressure of a solution on the excitability of a nerve, which is immersed in it, is an important part of the general physiology of osmosis. There are two hypotheses in regard to it. The one is that a hypertonic solution, which brings on a decrease of water in the nerve, causes an increase of its excitability, while a hypotonic solution, which brings on an increase of water in it, causes a decrease of its excitability. The other hypothesis states just the opposite, namely, that a hypertonic solution causes a decrease, while a hypotonic solution causes an increase of excitability of the nerve.

Eckhard (1), Kölliker (2), Birkner (3), Schiff (4) and Harless (5) observed that drying a motor nerve or withdrawing its water by means of a concentrated solution of neutral salts, urea or sugar, etc., causes an irregular excitation with clonic contractions or tetanus of the attached muscle, though both excitability and conductivity of the nerve disappeared after a certain period of time. This was confirmed by many workers. Birkner and Harless also stated that drying a nerve increased its excitability before the appearance of the clonic contractions. The latter entertained an opinion that the drying did not act as a direct stimulus to the nerve, but it caused an increased excitability shown by a muscular response to a stimulus lower than the threshold value, which stimulus, when applied to a normal nerve, gave no response at all. This hypothesis was experimentally proved by Ssubotin (6), Mandelstamm (7) and Buchner (8), while Limbourg (9) rather supported the view of Schiff, who maintained that the withdrawal of water itself acted upon the nerve as a direct stimulus. On the other hand Kölliker, Birkner, Ranke (10) and Härtl (11) proved

that a gradual decrease of excitability of the nerve took place at the time when it was immersed in water or dilute NaCl solution. Ranke recognized a temporary increase of excitability in the first stage of the process. Ishikawa (12) made many experiments and proved that the hypotonic solution decreased the excitability represented by the threshold strength of electrical stimulus, and that the hypertonic solution increased it, though it gradually decreased after the solution acted for a long time.

Durig (13) noted, on the contrary, a prolongation of latent period and a diminution of the propagation velocity of excitation in the nerve of a frog which had been kept in dried air so as to diminish the quantity of water in its body. Afterwards Urano (14) expressed the opinion, based upon his own experiments, that the excitability was increased by a hypotonic solution, while it was decreased by a hypertonic solution. This was supported by Renauld (15).

Nevertheless Laugier (16) measured the threshold strength of condensor discharge, applied as a stimulus to a nerve which was immersed in a hypotonic or hypertonic solution, and found that the value of b of Hoorweg's formula, $V = \frac{a}{C} + bR$, increased, and the value of a/b decreased in both cases.

The above is an outline of the work hitherto done on the effect of osmotic pressure on the nerve.

It is interesting to determine which of these hypotheses is correct. The first step to be taken is to examine the methods and results of the experiments which led to these antagonistic hypotheses.

Harless, who is known as the originator of the first hypothesis, made his assumptions without experimental proof. As far as is known, however, Ssubotin was the first who tested this hypothesis experimentally. He immersed the nerve-trunks of nerve-muscle preparations in concentrated solutions of NaCl, $NaNO_3$, glycerol, urea and sugar, and found that, before the appearance of the clonic muscular contractions, the excitability was increased, which was represented by the threshold strength of electrical stimulus. Buchner also measured the threshold strength of electrical stimulus to estimate the excitability of a nerve immersed in saturated solutions of NaCl and urea. Härtl did the same, immersing the nerve in water. Ishikawa measured the effects of various hypotonic and hypertonic solutions, each containing the same quantity of NaCl but differing in the content of sugar, on the excitability of the nerve represented by the threshold strength of single induction shock.

The second hypothesis is based on the experiments of Urano and Renauld. Urano immersed a nerve in solutions half as concentrated, and twice as concentrated as the normal Ringer's solution alternately, and measured the threshold stimulus of a break, constant current, single induction shock and the threshold of mechanical stimulus. Renauld preferred the discharge of a condensor as stimulus, but he applied a method of an artificial circulation to the hind legs of the frog so as to test the effects of hypotonic and hypertonic solutions. The results of his experiments, however, necessarily were complicated by the effects of the solutions on the neuromuscular junctions and muscle also.

Now two of the above experiments can be taken as representatives of the two antagonistic hypotheses,—the experiment of Ishikawa for the first and that of Urano for the second.

In the present work, a series of these two experiments was repeated by the writer. Then the method of estimating the excitability of the nerve was examined and it was found that the threshold strength of electrical stimulus could not represent the excitability of a nerve in this case. The effects of hypotonic and hypertonic solutions were determined by means of measuring the propagation velocity of excitation through an affected nerve.

TEST OF URANO'S AND ISHIKAWA'S EXPERIMENTS

The methods taken in the experiments of Urano and Ishikawa agree with each other in using a nerve-muscle preparation of a frog as the material, and also in measuring the threshold strength of a break, single induction shock, to estimate the excitability of the nerve, but differ from each other in the following two points.

1. Urano used as the hypotonic solution a Ringer's solution made half as strong as the ordinary Ringer's solution, and as the hypertonic solution a Ringer's solution, made twice as strong as the ordinary one. Ishikawa, however, in all his solutions, used the same concentration of NaCl, that just sufficient to maintain the activity of the nerve, and made these solutions either hypotonic or hypertonic by adding various quantities of saccharose to them.

2. Urano immersed the nerve-trunk in his hypotonic and hypertonic solutions alternately for four hours at a time, and measured its excitability at the end of each period. Ishikawa, however, put a part of the nerve-trunk in his specially prepared vessel containing the solu-

tion, and measured the changes of its excitability with respect to time during the period of immersion.

In the present research, the solutions prepared according to Urano and Ishikawa were used separately, so as to repeat their experiments, but the latter's method was preferred to the former's, for it was then possible to better study the changes of excitability of the nerve at intervals while the solution was acting.

As the material of the experiments, a sciatic nerve of a bull-frog (Bufo vulgaris variata japonica) with gastrocnemius muscle attached was used. A part of the nerve was put in a vessel prepared according to Ishikawa. This vessel is a glass tube which has two small holes each on the opposite wall. The nerve-trunk was put through these holes and the space between the nerve and hole was plugged with vaseline to prevent the solution from leaking out. The solution was poured into the vessel. The nerve was touched by three pairs of platinum

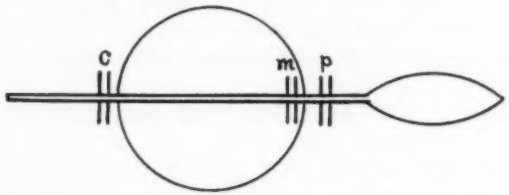

Fig. 1. A nerve-muscle preparation in vessel; *c*, *m* and *p* are platinum electrodes.

electrodes, which were alternately used. One pair (*m* in fig. 1), which were in the tube, made contact with the nerve near the side of the vessel which was opposite the muscle. This electrode was used to test the excitability of the affected part of the nerve. The other two pairs were put outside the tube; the one (*c* in fig. 1) touching the central part of the nerve and the other (*p* in fig. 1) touching the peripheral part of the nerve. The former was used to test the conductivity of the affected part of the nerve while the latter was used to test the indirect irritability of the attached muscle. When the muscle showed any change in irritability as was elicited by the last electrode, it was thrown out of the series. With respect to this method, see also Ishikawa's paper (12).

After the nerve-muscle preparation was dissected out it was preserved in Ringer's solution for one hour and then put into the vessel as prepared above. It was then allowed to remain here for two hours so

as to recover from any mechanical influence on its excitability. This latter procedure was advocated by Fröhlich (17). The experiment was then commenced.

The part of the preparation outside the vessel was covered with cotton soaked with Ringer's solution. The cotton was taken off at the time of each measurement. The whole apparatus was put in a moist chamber.

When measuring the excitability, the solution was removed and all the drops of the solution attached around the nerve and the electrodes were wiped off with a hair-pencil, so that a short circuit might not occur.

The stimulating current was supplied by an induction coil. The primary was connected in series with an accumulator, a wire-resistance and a platinum-mercury key. The secondary coil was connected with any one of the three pairs of electrodes of the vessel by means of Pohl's commutators without cross wires. A break induction shock was applied to the nerve in the descending direction.

The concentrations of the physiological saline and of isotonic dextrose solutions were as follows.

Ringer's solution: 0.67 gram NaCl, 0.02 gram KCl, and 0.02 gram $CaCl_2$ (dried) in 100 grams water.

Physiological NaCl solution: 0.70 gram NaCl in 100 grams water.

Isotonic dextrose solution: 4.0 grams dextrose (without water of crystallization) in 100 grams water.

All these solutions depress the freezing point equally, i.e., $\Delta = -0.414°C$. The solvent of the solutions was water distilled through a glass or tin worm.

Two kinds of hypertonic solutions were used, namely Urano's and Ishikawa's. The former has n times the concentration of saline solutes, as the physiological saline solution, and for the sake of convenience the writer calls this "n × saline solution," while the latter is prepared by dissolving n−1 times as much dextrose as is present in the isotonic solution in the physiological NaCl solution, which the writer calls "Ishikawa's n × solution" or "NaCl + (n−1) × dextrose solution." The osmotic pressure of the physiological solution is called "the physiological pressure."

The experiments were carried on in the two succeeding winters of 1914 to 1915 and 1915 to 1916, in a room without heat, so that the muscle-nerve preparations might keep their activity for as long a time

as possible. It was found that the nerve immersed in the physiological NaCl solution kept its excitability unchanged for fifty hours at 8 to 10°C., and for more than twenty hours when at 14 to 15°C., and for a longer time when the nerve was immersed in Ringer's solution. The experiments for this paper then were never continued longer than this time at the temperature noted.

The results of the experiments show the following facts. The 2 × NaCl and 2 × Ringer's solution gradually decreased the threshold strength of the electrical stimulus, agreeing here with Urano's experiments, while the 1.3 × and 1.5 × NaCl solution caused a slight and irregular change of the threshold strength. Experiments 1 and 2, out of seven observations made, will serve as examples.

Experiment 1. *Effect of 2 × Ringer's solution. Temperature 8.8 to 10.6°C.*

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
2 × Ringer's solution	0	0	44.0
		5	42.5
		10	41.5
		20	41.0
		40	39.0
	1	0	37.5
	1	40	34.0
	2	20	33.5
	3	20	33.0
	4	0	33.0
Ringer's solution		5	34.5
		10	35.5
		20	36.0
		40	39.0
	1	10	39.5
	3	0	39.5
	7	0	42.0

On the other hand, Ishikawa's 2 × and 3 × solutions (NaCl + 1 × dextrose and NaCl + 2 × dextrose solution) surely increased the threshold strength, but after several hours gradually decreased it, both results agreeing with those of Ishikawa. Six observations were made, one of which is shown in experiment 3.

Experiment 2. Effect of 1.3 × NaCl solution. Temperature 6.2 to 7.8°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
0.9 per cent NaCl solution	0	0	47.5
		10	48.0
		30	47.0
	1	0	46.0
	1	50	46.0
	3	0	48.0
	5		48.0
	8		47.5
	17		47.5
Physiological NaCl solution		30	50.0
	1	30	49.0
	4		48.0

Experiment 3. Effect of NaCl + 1 × dextrose solution. Temperature 6.0 to 8.2°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
NaCl + 1 × dextrose solution	0	0	39.5
		10	42.0
		30	44.5
		50	44.5
	1	10	44.5
	1	40	43.0
	2	20	42.5
	3	30	42.5
	4	30	42.5
	5	0	43.0
	15		38.5

As the hypotonic solution both Urano and Ishikawa used diluted saline solutions, but obtained quite different results. The result of the writer's own observations, however, differed somewhat from both these observers. The $\frac{3}{4}$ NaCl solution showed no remarkable change of the threshold strength of electrical stimulus. The $\frac{1}{2}$ saline solution in most cases showed an increase of the threshold strength at first and then a decrease of it; while in some cases a decrease was observed from the beginning; again in another case, no remarkable change was appar-

ent at all. The $\frac{1}{4}$ NaCl solution increased the threshold value at first for a short period and then decreased it remarkably. Examples of these cases are to be found in the following experiments 4 and 5, taken from a series of eight observations.

Experiment 4. Effect of $\frac{1}{2}$ NaCl solution. Temperature 6.8 to 9.0°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
0.35 per cent NaCl solution	0	0	48.5
		10	53.0
		30	54.5
	1	0	55.0
	1	40	54.5
	2	10	54.5
	4		53.5
	6		52.0
	10		49.5
	18		43.5
	23		40.0
Physiological NaCl solution	2		45.0

Experiment 5. Effect of $\frac{1}{4}$ NaCl solution. Temperature 7.0 to 9.0°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
0.18 per cent NaCl solution	0	0	45.5
		10	46.0
		20	46.0
		40	46.0
		40	48.5
	3		52.5
	4		53.5
	8		48.0
	14		43.0
	19		37.0
	23		33.5
Physiological NaCl solution		20	34.5
	1		36.5
	3		37.0
	10		41.0

But it is possible that the effects of the diluted saline solutions mentioned above are due to the inequality of ionic concentration. To avoid this, the nerve-trunk was immersed, in the following experiments, at first in an isotonic $\frac{1}{2}$ saline + $\frac{1}{2}$ dextrose solution for 3 to 10 hours, until an equilibrium of ionic concentration was obtained, and then it was immersed in a $\frac{1}{2}$ saline solution to allow a lower osmotic pressure to affect the nerve, independently of the inequality of ionic concentration. These experiments show that there is almost no change of threshold strength of electrical stimulus in the isotonic solution, but a gradual decrease of it is seen in the $\frac{1}{2}$ saline solution. In the case of an isotonic $\frac{1}{4}$ saline + $\frac{3}{4}$ dextrose solution being applied at first to the nerve and then $\frac{1}{4}$ saline solution, almost the same result is obtained as above except an initial increase of the threshold strength caused by the isotonic solution. Experiment 6 is quoted here out of seven observations made.

Experiment 6. Effect of $\frac{1}{2}$ NaCl solution. Temperature 7.0 to 10.2°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
	0	0	38 5
		20	39.0
		40	39.5
$\frac{1}{2}$ NaCl + $\frac{1}{2}$ dextrose solution	1	0	39.5
	1	20	39.0
	1	40	38.5
	2	0	39.0
	4	0	39.0
		20	39.0
	1	0	38.5
	1	40	38.0
$\frac{1}{2}$ NaCl solution	2	20	36.5
	3	20	35.5
			33.0
	13		23.0
	1		23.5
$\frac{1}{2}$ NaCl + $\frac{1}{2}$ dextrose solution	2		32.0
	3		33.0

The results stated above make the writer skeptical as to whether or not the excitability of the nerve is really represented by the threshold strength of electrical stimulus.

CAN THE EXCITABILITY OF THE NERVE BE REPRESENTED BY THE THRESHOLD STRENGTH OF ELECTRICAL STIMULUS?

It has been the common method to estimate the excitability of nerve by measuring the threshold strength with electrical induction shocks applied as a stimulus to the nerve. The popularity of this method is due chiefly to the readiness of its treatment and the exactness of its results. But special care must be taken in adopting this method when measuring the effect of osmotic pressure on the nerve. The reason is this:

Tanemura (18) showed that the electrical resistance of a nerve immersed in a hypotonic or hypertonic solution varied widely according to the increase or decrease of its saline contents, and that such a variation of the electrical resistance caused a deviation of the threshold strength of electrical stimulus. It was also found by the writer (19) that, in the case of the isotonic sugar solution, the variation of electrical resistance of the nerve made it impossible to estimate its excitability by the threshold strength of electrical stimulus, and that the excitability, in such a case, could be represented by the threshold strength of mechanical stimulus or the propagation velocity of excitation through the affected part of the nerve. Judging from these experiments it is quite certain that the threshold strength of electrical stimulus of the nerve, which was acted on by a hypotonic or hypertonic solution, implies an error due to the variation of electrical resistance of the nerve. Therefore the threshold strength of electrical stimulus does not represent the real excitability of the nerve, that is to say, the induction current which passes through the nerve is deformed by the variation of electrical resistance of the nerve, and accordingly the stimulating factor of the current is changed.

For this reason, in studying the effect of osmotic pressure on the excitability of the nerve, a method for measuring the excitability should be adopted which will not be influenced by any variation of electrical resistance of the nerve. In the writer's former report (19) it was stated that the threshold strength of mechanical stimulus or the propagation velocity of excitation gave exact results for the excitability in the case of the isotonic sugar solution. In the present research, however, it was found that the threshold strength of mechanical stimulus showed irregular variations in many experiments, though the method was just like the former one. This might be due partly to the change of the thickness of the nerve depending on the con-

centration of the solutions in which the nerve was immersed. Consequently there is only one method to be used, that is, the measurement of the propagation velocity of excitation in order to estimate the excitability of the nerve affected by the change of osmotic pressure.

MEASUREMENT OF THE PROPAGATION VELOCITY OF EXCITATION THROUGH A NERVE

It is well known that the local excitation at a stimulated point and the propagation wave of excitation which passes through this point depend upon the same physico-chemical reaction in the nervous element (20). That is to say, the excitability and propagation velocity are two functions of the same reaction, and they vary parallel with each other under changing conditions. Hence it follows that the excitability of a nerve at a given point can be estimated by measuring

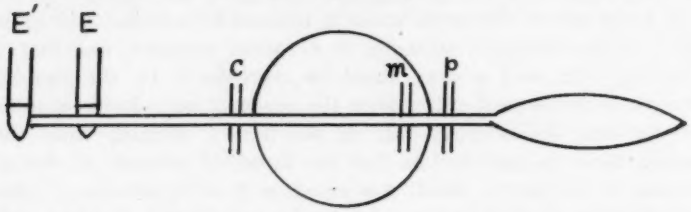

Fig. 2. The arrangement of electrodes for recording the action current; *c*, *m* and *p* are platinum electrodes. *E* and *E′* are a pair of non-polarizable electrodes, which lead off the action current to the galvanometer.

the propagation velocity of the excitation wave which passes through that point. This is the only reliable method of estimating the excitability of a nerve affected by osmotic pressure, as has already been pointed out.

To measure the propagation velocity, the nerve is stimulated at two points, one at the central end and the other at the peripheral end of the affected part, and the velocity is calculated from the difference between the latent periods of the action current caused by the same stimulus given to each point. An Einthoven's string galvanometer is used to record the action current. The arrangement of the apparatus has already been described in a former report (19). Refer also to the figure 2 and the explanation of figure 3. A quartz string of 2300 ohm resistance was used which was drawn as tight as possible but not tight

enough to produce any self-oscillation The velocity thus calculated might imply an error of 4 to 6 per cent.

In each of the various experiments, the threshold strength of electrical stimulus was also measured, for the sake of comparison, at the same time when the velocity was taken.

There is a difference in the excitability of nerve in reference to the threshold strength of electrical stimulus and the propagation velocity of excitation The latter measured by the method mentioned above is the mean velocity of all fibers of a nerve-trunk, while the former is the excitability of a few fibers, though theoretically a single fiber is desired. When a nerve-trunk is immersed in a solution, the outer layers of the trunk are affected by the solution more rapidly than the inner layers.

It was seen that, when a nerve is immersed in physiological saline solution, there is no change in the propagation velocity for fifty to sixty hours at 8 to 10°C. and for twenty-four hours at 15°C. The series of experments here reported was then made within these limits of time. The temperature was kept as low as possible, for the higher the temperature, the greater the velocity becomes, and accordingly the error of measurement increases.

The results of the experiments are as follows:

1. The effect of hypertonic solutions. At first Urano's hypertonic solution was used. When a nerve was immersed in a 2 × NaCl solution, the propagation velocity gradually decreased during the first several hours until an equilibrium was established. When the hypertonic solution was replaced by a physiological saline solution, the velocity was gradually regained. In 1.5 × and 1.75 × NaCl solution, similar results were obtained with a slight change of the velocity. Seven experiments were done with the same results, one of which is given in experiment 7.

In a 4 × NaCl solution, the velocity decreased more rapidly than in the former case and the action current became weaker and weaker until it disappeared as the nerve fibers gradually lost their conductivity. When the solution was replaced by a physiological salt solution, the nerve recovered its conductivity and the velocity of propagation was increased. In a 6 × NaCl solution, the velocity decreased still more rapidly and the action current disappeared in a shorter time. In a 7 × NaCl solution, the action current disappeared after 15 minutes. In such a short time it was impossible to examine the rate of decrease of the propagation velocity. By way of example, an experiment with 4 × NaCl solution is shown in experiment 8.

Experiment 7. Effect of 2 × NaCl solution. Temperature 7.0 to 9.0°C.

SOLUTION	TIME	THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours		
2 × NaCl solution	0	49.5	16.5
	1	48.5	
	4	48.0	8.5
	5	49.0	
	6	49.0	
	8	48.5	8.2
	10	48.0	
	13	46.0	7.9
	23	41.5	7.6
Physiological NaCl solution	0.5	44.5	
	1	44.5	9.5
	2	44.5	
	4	43.5	
	5	44.5	11.9
	9	45.5	12.4
	13	46.0	13.5
	19	46.5	13.8

Experiment 8. Effect of 4 × NaCl solution. Temperature 6.5 to 8.0°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours	*minutes*		
4 × NaCl solution	0	0	43.5	13.2
		40	39.5	13.0
	1	5	Contractions	
	1	10	Contractions	10.4
	1	40	Contractions	
	1	50	39.0	7.0
	3	0	38.5	3.5
	5	0	35.5	—
Physiological NaCl solution	1		40.0	3.5
	3		42.0	8.1
	6		43.0	9.3
	11		43.5	11.4

In a more concentrated solution than 4 × NaCl, it was found that an excitation of the nerve occurs after the nerve is immersed a certain time. This is indicated by spontaneous clonic contractions or a tetanus. This makes the measurement of the threshold strength of electrical stimulus impossible. When these contractions occur, the galvanometer shows no action current so that the measurement of the propagation velocity is not impeded. The reason why the galvanometer shows no action current is this: The excitation of each nerve fiber occurs at a different time, so that both phases of the action currents in different fibers are neutralized by each other, thus making it impossible for the currents to be united and become of measurable intensity.

Next Ishikawa's hypertonic solution was used. In his 2 × solution (NaCl +1 × dextrose solution), the velocity began to decrease gradually from the beginning of the action of the solution, but slower than in the case of 2 × NaCl solution. When the solution was replaced by a physiological salt solution, the velocity was regained gradually. In his 1.75 × solution (NaCl + 0.75 × dextrose solution), a little decrease of the velocity was seen, while in the 1.25 × solution (NaCl + 0.25 × dextrose solution) and in the 1.5 × solution (NaCl + 0.5 × dextrose solution), its decrease was so slight that it might be regarded as an experimental error. Seven experiments were made, all with the same results, one of which is shown in experiment 9.

Experiment 9. Effect of Ishikawa's 2 × solution. Temperature 8.0 to 9.3°C.

SOLUTION	TIME	THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours		
NaCl + 1 × dextrose solution	0	47.0	16.8
	1	46.5	16.2
	2	48.5	15.7
	4	50.0	14.1
	7	51.0	13.1
	12	52.0	11.8
Physiological NaCl solution	1	47.5	11.9
	3	43.0	13.1
	7	42.5	13.1
	11	43.5	14.7
	17	44.5	17.8

In Ishikawa's 4 × solution (NaCl + 3 × dextrose solution), the velocity decreased rapidly. In the 6 × solution (NaCl + 5 × dextrose solution), it decreased still more rapidly and the action current became weaker and weaker until after several hours it disappeared altogether, thus making the measurement of the velocity impossible. In the 7 × solution (NaCl + 6 × dextrose solution), the action current disappeared after an hour and at the same time clonic contractions appeared in the muscle. By way of example an experiment with 6 × solution is shown in experiment 10.

Experiment 10. Effect of Ishikawa's 6 × solution. Temperature 11.8 to 13.0°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours	*minutes*		
NaCl + 5 × dextrose solution	0	0	49.5	18.3
		5	48.5	
		15	48.5	
		30	49.0	
		45	50.0	
	1	0	52.5	17.7
	1	20	53.5	
	1	40	54.0	
	2	0	55.5	17.1
	2	30	55.5	
	3	0	55.5	14.1
	3	30	55.5	
	4	0	56.0	10.1
	5	0	56.5	
	6	0	53.0	——
	8	0	52.5	——
Physiological NaCl solution		5	49.5	——
	1	0	48.5	4.9
	1	30	50.0	8.9
	5	30	48.0	15.0
	10		48.0	15.2
	15		51.0	17.2

In short, the propagation velocity of excitation of the nerve gradually decreases when the nerve is immersed in any hypertonic solution. This decrease of the velocity has no relation to the variation of the threshold strength of electrical stimulus. The higher the osmotic pressure, the

more rapidly the velocity decreases. The effect of a concentrated NaCl solution is stronger than that of a NaCl + dextrose solution of the same osmotic pressure. This is to be expected considering the difference of diffusion velocity of salt ions and dextrose molecules.

2. The effect of hypotonic solutions. In the ½ saline solution the propagation velocity of excitation gradually decreased, quite apart from the variation of the threshold strength of electrical stimulus. When the solution was replaced by a physiological salt solution, the velocity was, in some cases, restored to a certain degree, while in other cases it was not. Experiment 11, taken from eleven experiments, illustrates these points.

Experiment 11. Effect of ½ Ringer's solution. Temperature 9.9 to 11.0°C.

SOLUTION	TIME	THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours		
½ Ringer's solution	0	37.0	17.2
	2	37.5	
	4	36.0	15.6
	8	36.5	11.1
	12	35.0	
	14	34.5	8.5
	22	33.5	7.1
Ringer's solution	2	35.5	
	4	37.0	10.7
	8	36.5	11.0
	13	38.0	11.9
	19	37.0	11.8
	32	34.5	11.8

In the ⅓ or ¼ saline solution, the velocity decreased still more rapidly than in the first case and the conductivity disappeared after a certain time. When the solution was replaced by a physiological solution, the original value for the velocity was not regained but some indications of restoration were seen by the reappearance of the velocity which had previously disappeared. Experiment 12 will serve as an example.

Experiment 12. Effect of ¼ NaCl solution. Temperature 7.0 to 8.5°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours	*minutes*		
¼ NaCl solution	0	0	42.5	12.3
		20	45.5	12.2
	1		44.5	
	3		46.5	10.1
	5		50.0	8.0
	7		44.5	—
	8		44.5	—
Physiological NaCl solution		30	46.0	—
	2		42.0	6.7
	4		44.0	6.8
	8		45.5	6.1
	14		42.5	8.1
	20		44.5	7.9

Next the effect of the lower osmotic pressure was studied which did not imply a condition of inequality of ionic concentration. It was first seen that, in the isotonic ½ NaCl + ½ dextrose solution or ¼ NaCl + ¾ dextrose solution, the velocity decreased a little and then attained an equilibrium after 5 to 7 hours. The result of six experiments done confirmed this. Then, when a nerve was immersed in an isotonic ½ NaCl + ½ dextrose solution and after the equilibrium state of the velocity was attained, the solution was replaced by a ½ NaCl solution. The result was a gradual decrease of the velocity without any indication of its increase. The same procedure of experimentation was adopted in reference to another set of solutions, namely, isotonic ¼ NaCl + ¾ dextrose solution and ¼ NaCl solution, with the same result. But the decrease of the velocity in the latter series of experiments was so rapid that after five hours the nerves lost their conductivity. When the hypotonic solution was replaced by the isotonic solution, a certain degree of the velocity was restored. Experiments 13 and 14, taken from five experiments, will serve as examples.

Experiment 13. Effect of ½ NaCl solution. Temperature 14.0 to 14.5°C.

SOLUTION	TIME	THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours		
½ NaCl + ½ dextrose solution	0	55.0	20.5
	2	56.5	
	4	57.0	20.0
½ NaCl solution	0.5	57.0	
	1	57.0	20.2
	2	57.0	
	4	56.5	
	9	54.0	13.3
	15	52.0	11.3
½ NaCl + ½ dextrose solution	2	55.0	
	4	57.0	11.5
Physiological NaCl solution	1	58.0	
	2	59.0	12.8

Experiment 14. Effect of ¼ NaCl solution. Temperature 14.0 to 15.8°C.

SOLUTION	TIME	THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours		
¼ NaCl + ¾ dextrose solution	0	46.0	21.6
	0.5	46.0	
	7	47.5	18.1
¼ NaCl solution	0.5	47.5	
	1	48.5	
	1.5	49.5	
	2	50.5	13.1
	3	50.5	
	4	52.0	6.9
	8	46.0	—
¼ NaCl + ¾ dextrose solution	1	46.0	—
	2	46.0	—
	4	44.5	10.0
	6	44.5	10.6

In a word, an osmotic pressure lower than the physiological gradually decreases the propagation velocity of excitation independent of the variation of the threshold strength of electrical stimulus. The lower the osmotic pressure, the more rapidly the velocity decreases.

To sum up the results of these experiments we can state that the propagation velocity of excitation through the nerve is at a maximum when the nerve is immersed in a solution of physiological osmotic pressure. It becomes less in accordance with a decrease or increase of the osmotic pressure below or above that of the physiological. Since the excitability of the nerve and the propagation velocity of excitation through it go parallel, the physiological osmotic pressure is the optimum pressure for the excitability of the nerve, and a lower or higher osmotic pressure than the physiological decreases its excitability in accordance with the amount of the deviation from the normal.

Fig. 3. The plate shows two kinds of experiments for measuring the propagation velocity of excitation by means of recording the action current: experiment 9 denotes the effect of a hypertonic solution (NaCl + 1 × dextrose solution), and experiment 11 denotes the effect of a hypotonic solution (½ Ringer's solution). Figures of the *c* series in each experiment show action currents when the stimuli are applied on the electrode *c* in figure 2. Figures of the *p* series show other action currents when the stimuli are applied on the electrode *p* in figure 2. The two white vertical lines on each figure are artificially drawn to facilitate distinguishing the moment of stimulation and the beginning of the action current. The distance between these two lines represents the time in which the action current travels from the stimulating electrodes *c* or *p* to the leading-off electrode, *E*, which runs to the galvanometer. The difference between these distances on each pair of figures of the same number represents the time of propagating the current through the nerve from the electrode *p* to the electrode *c*. The real distance from *p* to *c* is 42.5 mm., and the affected part of the nerve in the vessel is 32.5 mm. Therefore the sum of the length of the part of the nerve which lies between *p* and *c* but outside the vessel is 10.0 mm. It was assumed that the propagation velocity through this part of the nerve was unchanged through the course of the experiment, and then the propagation velocity through the affected part of the nerve was calculated. The wave line below the figure of the action current is the figure of vibration of a tuning fork, one period of which corresponds to $\frac{1}{125}$ seconds. The values of the velocity thus calculated are given in the following tables.

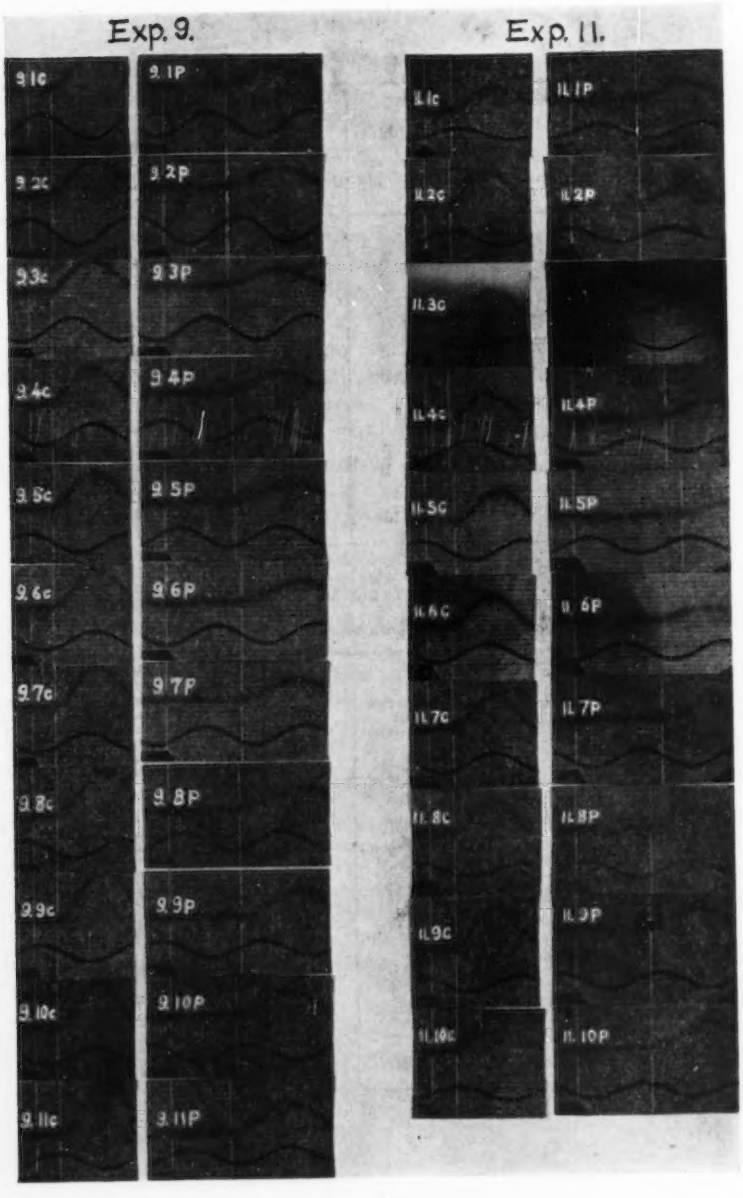
Exp. 9.
9.1c
9.1P
9.2c
9.2P
9.3c
9.3P
9.4c
9.4P
9.5c
9.5P
9.6c
9.6P
9.7c
9.7P
9.8c
9.8P
9.9c
9.9P
9.10c
9.10P
9.11c
9.11P
Exp. 11.
11.1c
11.1P
11.2c
11.2P
11.3c
11.4c
11.4P
11.5c
11.5P
11.6c
11.6P
11.7c
11.7P
11.8c
11.8P
11.9c
11.9P
11.10c
11.10P

Fig. 3

TABLE 1

Experiment 9

SOLUTION	NUMBER OF FIGURES	TIME	TIME OF PROPAGATION BETWEEN p AND c ($\frac{1}{1000}$ SEC.)	TIME OF PROPAGATION THROUGH THE AFFECTED PART ($\frac{1}{1000}$ SEC.)	PROPAGATION VELOCITY (M/SEC.)
		hours			
NaCl + 1 × dextrose solution	1	0	2.53	1.93	16.8
	2	1	2.60	2.00	16.2
	3	2	2.67	2.07	15.7
	4	4	2.91	2.31	14.1
	5	7	3.08	2.48	13.1
	6	12	3.35	2.75	11.8
Physiological NaCl solution	7	1	3.33	2.73	11.9
	8	3	3.08	2.48	13.1
	9	7	3.08	2.48	13.1
	10	11	2.82	2.22	14.7
	11	17	2.43	1.83	17.8

TABLE 2

Experiment 11

SOLUTION	NUMBER OF FIGURES	TIME	TIME OF PROPAGATION BETWEEN p AND c ($\frac{1}{1000}$ SEC.)	TIME OF PROPAGATION THROUGH THE AFFECTED PART ($\frac{1}{1000}$ SEC.)	PROPAGATION VELOCITY (M/SEC.)
		hours			
½ Ringer's solution	1	0	2.47	1.89	17.2
	2	4	2.66	2.08	15.6
	3	8	3.51	2.93	11.1
	4	14	4.40	3.82	8.5
	5	22	5.16	4.58	7.1
Ringer's solution	6	4	3.63	3.05	10.7
	7	8	3.53	2.95	11.0
	8	13	3.32	2.74	11.9
	9	19	3.34	2.76	11.8
	10	32	3.34	2.76	11.8

SUMMARY

1. The change of electrical resistance of the nerve which is immersed in a hypotonic or hypertonic solution makes it impossible to estimate the excitability of the nerve by measuring the threshold strength of electrical stimulus. The excitability can only be determined by the propagation velocity of excitation through the affected part of the nerve.

2. The optimum osmotic pressure for the propagation velocity of excitation, and accordingly for the excitability of the nerve is $\Delta = -0.4°C.$, which is the osmotic pressure of 0.7 per cent NaCl solution (about 5 atmospheres of pressure at 0°C.). This might well be called the physiological osmotic pressure. A higher or lower osmotic pressure than this decreases the propagation velocity of excitation, and in consequence the excitability of nerve.

In conclusion the writer wishes to express his thanks to Prof. H. Ishikawa for direction and help during the course of this research.

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A PHYSIOLOGICAL STUDY ON THE LUMINESCENCE OF WATASENIA SCINTILLANS (BERRY)

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INTRODUCTION

Watasenia scintillans (Berry), a luminous cephalopod, is found along the northeastern coast of Toyama-Bay in the Japan Sea. It is commonly called "Hotaru-ika" in our national language meaning literally firefly-squid.

Up to the present time what is called Abraliopsis, a genus of luminous cephalopods, has been believed to be very rare, and in fact only a few samples have been collected. The studies of this animal were made by L. Joubin (1), W. Hoyle (2) and C. Chun (3). Several years ago S. Watase (4) reported large numbers of a species of this genus occurring in the sea mentioned above, and named it Hotaru-ika. Its morphology has since been studied independently by S. S. Berry (5), C. Ishikawa (6), M. Sasaki (7) and S. Matsuno (8). Berry named it Abraliopsis scintillans, but Ishikawa stated a few years later that it did not belong to this genus, and named it Watasenia scintillans (Berry) after the name of its discoverer.

This animal is a small squid with a mantle-length of 6 cm. Like Abraliopsis it has three kinds of luminous organs which Watase called the luminous organs of the first, second and third classes. The luminous organ of the first class, or the brachial organ, is the largest of all. It is at the end of each fourth arm and consists of three oval globules arranged in a series. The luminescence of this organ is stronger than the other two. The luminous organ of the second class, or the ocular organ, consists of five small organs which are arranged in a series along the ventral circumference of each eyeball. The luminous organ of the third class, or the cutaneous organ, consists of numerous minute organs dotted about on the ventral surface of the whole body. The microscopical structure of these luminous organs is nearly the same as

that of Abraliopsis morisii Vérany, which was described by Chun. Joubin and Chun were already aware of the light-producing function of the ocular and cutaneous organs, though they had not examined the living animal. The function of the brachial organ was not known until Watase observed its luminescence in the living animal.

The writer of this paper made physiological researches upon the luminescence of this animal in May and June of 1915 and 1916 at Uwozu, a town situated on the coast of Toyama-Bay.

THE EFFECT OF CONCENTRATION OF SEA WATER ON THE LIFE OF WATASENIA SCINTILLANS (BERRY)

Fishermen who catch this squid speak of it as being so delicate an animal that it lives only a very short time if it is put in a pail containing the sea water of the uppermost layer. It occurred to the writer that this might be due to a lower salinity of the uppermost layer near the coast rather than that of deep sea water. To test this idea the following experiments on the effects of concentration of the sea water on the life of this animal were made.

In order to compare the effects of sea water from various levels on the life of the animal, one squid was placed in 2 liters of sea water and left until it died. A record of the average time obtained in several experiments of the same kind was taken. Care was taken not to allow the ink spouted by the animal to become mixed with the sea water, for it is very injurious to the life of the animal.

The relation between length of life of the animal and the concentration of the sea water at various depths was first determined. By measuring the value of the osmotic pressure and the specific gravity, it was found that sea water below a depth of five meters has nearly the same concentration and has no injurious effect upon the life of the animal, while the sea water of the uppermost layer, which is relatively more dilute, shortens the animal's life. An example of experiments on this point is shown in table 1.

Next, the effects of the concentration of the sea water upon the life of the squid was estimated, using: *a*, deep sea water of 10 to 20 m., which was regarded as the standard solution; *b*, various dilutions of sea water made by adding distilled water; and *c*, various concentrations of sea water prepared by dissolving in it varying amounts of salt obtained from the deep sea water by evaporation. It was found that animals lived longest in the normal sea water, and a lesser time in

diluted sea water in proportion to the degree of dilution, especially in sea water more highly diluted than $\Delta = -1.5°C$. The same result as in the diluted sea water was obtained in a concentrated sea water but the shortening was not so marked. These experiments were repeated eight times. One series is shown in table 2.

Judging from the results shown in tables 1 and 2, it is obvious that the injurious action of the surface layer of sea water is due to its dilution. But this is not the only factor, for life should be lengthened in a solution prepared by dissolving the salt obtained from the deep sea water in either fresh water or sea water of an uppermost layer making

TABLE 1

Relation between the depth of the sea water and the length of life of Watasenia. Experimental temperature 18.1° to 18.8°C.

DEPTH OF THE SEA WATER	SPECIFIC GRAVITY AT 15°C.	FREEZING POINT DEPRESSION	CALCULATED CONCENTRATION OF ISOTONIC NaCl SOLUTION	MEAN LENGTH OF LIFE	
meters		*degrees C.*	*per cent*	*hours*	*minutes*
Uppermost layer	1.0125	−0.839	1.44		10
5	1.0254	−1.816	3.16	2	36
15	1.0260	−1.870	3.25	3	40
45	1.0261	−1.860	3.24	1	47
90	1.0260	−1.844	3.21		58
135	1.0267	−1.900	3.31	1	14
180	1.0266	−1.885	3.28	1	55

its concentration equal to that of deep sea water. But according to the writer's experiments life is remarkably longer in the latter than in the former.

Next it was attempted to estimate the effect of the dilution of sea water upon the osmotic pressure of the blood of Watasenia. But as this squid was too small to get a sufficient quantity of blood, some Ommastrephes were used in place of Watasenia in the following experiment. Several living Ommastrephes were placed in sea water of known osmotic pressure. After the death of the animals or after an hour in their living state, the blood was removed from the animal and its osmotic pressure was measured. The result of the measurement is as follows.

Freezing point depression of the sea water of 10 m. deep layer...−1.780°C.
Freezing point depression of the blood of the squids which are living in the above-mentioned deep sea water.....−1.796°C.
Freezing point depression of the sea water of the uppermost layer.....−0.537°C.
Freezing point depression of the blood of the squids which died in the same sea water of the uppermost layer.....−1.571°C.

TABLE 2

Relation between concentration of the sea water and the length of life of Watasenia. Specific gravity of 10 m. deep sea water, 1.0256 (at 15°C.). Experimental temperature, 19.5° to 21.0°C.

SOLUTION	FREEZING POINT DEPRESSION	CALCULATED CONCENTRATION OF ISOTONIC NaCl SOLUTION	MEAN LENGTH OF LIFE	
	degrees C.	*per cent*	*hours*	*minutes*
1.0 l. sea water + 1.0 l. water	−0.918	1.58		5
1.1 l. sea water + 0.9 l. water	−1.011	1.74		7
1.2 l. sea water + 0.8 l. water	−1.100	1.90		10
1.3 l. sea water + 0.7 l. water	−1.185	2.05		14
1.4 l. sea water + 0.6 l. water	−1.277	2.21		33
1.5 l. sea water + 0.5 l. water	−1.367	2.37		32
1.6 l. sea water + 0.4 l. water	−1.458	2.53		35
1.7 l. sea water + 0.3 l. water	−1.547	2.69	1	13
1.8 l. sea water + 0.2 l. water	−1.631	2.84	1	56
1.9 l. sea water + 0.1 l. water	−1.722	3.00	3	5
2.0 l. sea water	−1.813	3.16	3	0
2.0 l. sea water + 3 grams salt	−1.898	3.31	2	24
2.0 l. sea water + 6 grams salt	−1.982	3.46	1	6
2.0 l. sea water + 9 grams salt	−2.065	3.61	1	33
2.0 l. sea water + 12 grams salt	−2.150	3.76	1	5
2.0 l. sea water + 15 grams salt	−2.234	3.91	1	13

As is well known, the blood of a living squid has the same osmotic pressure as that of the sea water in which it lives. But this experiment shows that the osmotic pressure of the blood does not decrease parallel with that of the sea water when the latter becomes very low, and the animal dies when the osmotic pressure of the blood decreases to a certain degree. Lack of sufficient material prevented further investigation of this point.

Oxygen dissolved in the sea water is of course an important factor for maintaining the life of Watasenia. Three squids were placed in a specially equipped barrel containing 25 liters of deep sea water through which oxygen gas was passed against an inner pressure of two atmos-

pheres, so that the solubility of oxygen might be increased. Under these conditions one squid lived 18 hours; the other two 22 and 26 hours respectively. A control experiment was made under the same conditions differing only in keeping the inner pressure normal and without passing oxygen gas. In this case all three squids died after 6 hours. The results were confirmed by two other experiments of the same kind.

OBSERVATIONS OF THE LUMINESCENCE OF A LIVING WATASENIA

1. Observations of the luminescence of the luminons organs of the first class: Under natural conditions of living, the luminescence of the organs of this class is rarely seen. But as a consequence either of a mechanical or of any other stimulus, or when the sea water in which the animal is living becomes unsuitable for its life, the organs begin to function. The emission of light is periodical as in the case of the fire-fly; the duration time of one emission is not longer than 30 seconds and the interval varies considerably with varying conditions. Organs function equally well by day or by night. The light is bluish-white and shows a continuous spectrum, the extent of which could not be measured because of the type of spectroscope then available.

Upon the cessation of respiration or when the fourth arm is removed, this luminescent organ can no longer function. The following fruitless experiments were made to test this particular point: First the application of an electrical or mechanical stimulus directly upon the organ, indirectly upon the cut end of the arm, and then upon the pair of ganglia which are situated on the back of the head and are special to Watasenia. (This ganglion was detected by Prof. C. Ishikawa, and the writer is indebted to him for the information.) Stimuli applied to other parts of the body also gave negative results.

After the death of the animal the luminous organ is covered with pigment layers, but this can not be considered as the reason of the absence of luminescence for when the pigment cells contract so as to reveal the luminous substance, or when they are artificially removed, luminescence is no longer apparent.

2. Observations of the luminescence of the luminous organs of the second class: Observation of these organs which are situated on the ventral side of the eyeball is somewhat difficult in the living animal. To observe them the animal must be fixed on its dorsal side. But in this position the animal dies in a short time. Moreover it is not easy to

distinguish the luminescence of the organs of this class from that of the organs of the third class, which are scattered about in the skin over the eyeball. According to the writer's observations the luminescence of the organs of the second class is almost the same as that of the organs of the third class.

3. Observations of the luminescence of the luminous organs of the third class: Though the organs of this class are very numerous (about 600 on the mantle only), their illumination is so faint as to be just visible at a distance of one meter in a dark room. These ventrally located organs may be studied to advantage and with little disturbance to the animal by placing it over a mirror in a dish of sea water.

The intensity of the light varies from time to time and may almost disappear. Yet the luminescence cannot be said to be periodic as in the case of the organs of the first class, but is continuous. For instance, it was often observed that a single organ maintained its luminescence for more than 20 minutes. The color of the light of the organs is the same as that of the organs of the first class.

Within a short time after the death of the animal, the organs of the third class glitter with a blue light as seen by daylight, simulating the real luminescence. This condition was noticed by Joubin in the skin organs of Histioteuthis (9) and Abraliopsis. However, in the organs of Watasenia, many instances were observed in which the organs thus functioning in the daylight showed no luminescence when observed in a dark room. Again this glitter could not be seen from an oblique direction. We can infer from this phenomenon that it is not the real luminescence but perhaps only a reflection of the daylight from the organs, just as in the case of a cat's eyes which reflect in a dimly lighted room. This glitter of the organs disappears after a certain time and only numerous gray points are seen. But even in this state their luminescence can still be observed by application of a stimulus to the organs.

When a mechanical or electrical stimulus is applied to a living animal or to a dissected mantle, the organs of the third class illuminate strongly.

The dissected mantles were used to study the effects of various conditions upon the luminescence of this animal, because the organs of the first and second classes can not conveniently be used for this purpose.

ON THE LUMINOUS SUBSTANCES

As is well known, the bioluminescence is due to the oxidation of a certain substance produced by the cells of the luminous organs which are differentiated glands. According to R. Dubois (10) this oxidation is catalysed by a special oxidative enzyme produced by the organism. He extracted a luminous substance and an oxidative enzyme from the secretion of Pholas dactylus and named them luciferine and luciferase respectively. Recently E. N. Harvey (11) reported that the luciferase which he extracted from Cypridina and the firefly was not an enzyme, but the source of the light. H gave the names of photogenin for luciferase and photophelein for luciferin.

But the presence of such luminous substances cannot be experimentally proved in all kinds of luminous organisms. According to Pütter (12) tae bioluminescence is of two kinds, viz., extracellular and intracellular luminescence. In the animal in which extracellular luminescence takes place, the luminous organ still maintains the original structure of a gland, having both alveoli and ducts. The secretion is in this case oxidized so as to emit light soon after it is secreted in the cavity of the alveoli or after it is expelled from the gland. From a luminous organ of this kind the luminous substance can be extracted. In the animal in which intracellular luminescence takes place, the luminous organ is highly differentiated, alevoli and ducts being reduced to a mass of cells. The luminous substance is made to produce light in the cells as soon as it is produced, so that no secretion is expelled from them. As can be easily seen from this, it is very difficult, if not impossible, to extract the luminous substance from the organ of this class.

The luminescence of Watasenia belongs to the type of intracellular luminescence because the histological structure of the luminous organs shows neither alveoli nor ducts. Therefore the extraction of the luminous substance from this animal is undoubtedly very difficult. In fact several unsuccessful attempts were made to extract it with water and several organic solvents.

NECESSITY OF OXYGEN FOR LUMINESCENCE

Since bioluminescence is an oxidative process, oxygen is absolutely necessary. The luminescence of Watasenia may be taken as an example. According to the writer's observations, a dissected mantle emits light more strongly in air than in the sea water and most strongly in pure oxygen. It was observed that when a mantle was put in free

atmospheric air, its light disappeared altogether after 15 hours when its outer surface became dry, while another piece which was put in a glass vessel in order to prevent its quick drying (for this purpose a Verworn's gas chamber of about 10 cc. was used), kept its luminescence for more than 25 hours (at room temperature of 20 to 23°C.).

Again putting the third piece in a gas chamber and passing hydrogen or carbon dioxide gas through it, it was observed that the luminescence disappeared after 10 to 25 seconds. When these gases were replaced by air, the luminescence reappeared in from a few to some 20 seconds. When pure oxygen was used instead of air, the luminescence became more intense than in air. Such an experiment could be repeated with a single specimen as many times as one wished.

Consequently it may be stated that oxygen is indispensable for the luminescence of Watasenia.

THE EFFECT OF NARCOTICS ON LUMINESCENCE

It is said that in many luminous organisms narcotics first stimulate, then inhibit the bioluminescence. The effect of narcotics on Watasenia agrees with this statement. When mantles are placed in a Verworn's gas chamber and air saturated with vapor of alcohol, ether or chloroform is passed through it, the luminescence becomes more intensive at first, then gradually diminishes and after a certain time disappears. But if the narcotics are replaced by air, the luminescence reappears in a few seconds. The effect of ether is most injurious, chloroform next and alcohol least. But these differences may not be attributed to the nature of the narcotics, for the vapor pressures of these narcotics (at room temperature of 20°C., at which the experiments were made) are different, but parallel to the different intensities of their activity. The results of the experiments are shown in table 3.

TABLE 3

The effect of narcotics on the luminescence of Watasenia. Experimental temperature, 20°C.

NARCOTICS	TIME REQUIRED TO REACH THE MAXIMAL LUMINESCENCE	TIME REQUIRED FOR THE DISAPPEARANCE OF LUMINESCENCE	TIME REQUIRED FOR THE RECOVERY OF LUMINESCENCE IN AIR	SATURATED VAPOR TENSION OF NARCOTICS AT 20°C.
	seconds	*seconds*	*seconds*	*mm. Hg.*
Ether	1	2– 3	1–3	442.4
Chloroform	1.5– 3	6–10	1–3	160.5
Alcohol	5 –20	60–90	1–3	44.0

We may conclude, therefore, that narotics first excite then inhibit luminescence and that this inhibition is reversible.

THE EFFECT OF TEMPERATURE ON LUMINESCENCE

The results of many experiments on the effect of temperature upon bioluminescence are in complete agreement on the point that there is a limited optimal range of temperature for bioluminescence and that any

TABLE 4

The effect of temperature on the luminescence of Watasenia

TEMPERATURE	DURATION OF LUMINESCENCE		RECOVERY OF LUMINESCENCE WHEN RETURNED TO 26°C.
degrees C.	*hours*	*minutes*	
−12.0		2	Recover after 10 minutes
− 3.3		5	Recover after 2 minutes
10.0		25	Recover after 3 minutes
17.0 (16.0–18.0)	12		
20.1 (18.8–21.4)	13		
21.0 (20.0–22.0)	12		
24.0 (constant)	11		
25.0 (constant)	12		
28.7 (29.3–28.0)	13		
30.4 (31.8–29.0)	10		
31.6 (32.6–30.6)	5		
32.0 (32.5–31.5)	3		
33.6 (34.0–33.2)	1		Recover after 1 minute
35.0		30	Recover after 1 minute
36.0		16	Recover after 1 minute
39.0		10	Recover after 1 minute
40.8		2	Recover after 1 minute, but irreversible after 2 or 3 repetitions
44.5		1	Recover after 3 minutes, but irreversible after 2 repetitions
48.8		1	Irreversible, mantles coagulate
63.0	Immediately		Irreversible, mantles coagulate

temperature outside this range acts unfavorably upon it. This optimal range of temperature is variable according to the materials and to the method which is employed.

Experiments upon the effects of temperature on the luminescence of Watasenia were made, the results of which are shown in table 4. The following method was used. Dissected mantles were put in a test tube which was again put in a Dewar's flask (a thermos bottle) con-

taining water at a constant temperature. The flask was closed with a cork through which a thermometer was inserted. The inner temperature varied somewhat as the time passed on. The initial and the final temperature are given parenthetically in the table.

From these results it may be concluded that the optimal range of temperature for the luminescence of Watasenia is from 16°C. to 31°C. Within this range luminescence persists through about the same time interval but the intensity of the light is stronger at the lower temperature (16 to 20°C.) than at the higher. The inhibition of luminescence at a lower temperature than 10°C. (down to −12°C.) is perfectly reversible. The inhibition at 36 to 45°C. is also reversible but after repeated experiments or when heat is applied for a long time the luminous organ loses its reversibility. A higher temperature than 49°C. entirely destroys the illuminating power of the organ due to the heat coagulation of its cells.

THE EFFECT OF SUNLIGHT ON LUMINESCENCE

Almost all work hitherto published refers to the fact that the intensity of bioluminescence is diminished after the exposure of the animal or the luminous organ to sunlight or to strong artificial light. But the results obtained by the writer differ from this.

Many dissected mantles of Watasenia were exposed to the sunlight for from 20 minutes to 2 hours, taking precautions to prevent desiccation, but no difference in luminescence was observed between the materials which were brought into a dark room after exposing to the direct sunlight for even 2 hours and the materials which were kept in the dark room from the beginning.

THE EFFECT OF OSMOTIC PRESSURE ON LUMINESCENCE

It is well known that, when the luminous organ of an animal is dried or immersed in glycerol to withdraw its water, it loses completely the illuminating power. But with regard to the effect of immersing the organ in water, various results are reported by various workers. There is no information on the effect of osmotic pressure upon the bioluminescence, except the work of Kölliker and of Mangold. Kölliker (13) observed that the 12 to 20 per cent NaCl solution intensified the luminescence of Lampyris at first, and then after a short time paralyzed it. Mangold (14) observed that a concentrated NaCl solution intensified the luminescence of Ophiopsila but not that of Maurolicus.

Watasenia is favorable for testing the effect of osmotic pressure upon bioluminescence, for it lives in the sea. Some dissected mantles were put in the sea water of various concentrations and the effects of these solutions on luminescence were examined. The solutions were

TABLE 5

The effect of osmotic pressure on luminescence. Experimental temperature 16.2° to 18.8°C.

OSMOTIC PRESSURE OF SOLUTION (THE PRESSURE OF NORMAL SEA WATER BEING TAKEN AS 1)	CONCENTRATION OF ISOTONIC NaCl SOLUTION	TIME OF PERSISTENCE OF THE LUMINOUS POWER (IRRITABILITY TO THE STIMULUS)
	per cent	*hours*
3.7	11.14	1
3.2	9.74	1
2.3	6.96	1
2.0	6.06	1
($\triangle = -3.448$)		
1.9	5.76	2
1.8	5.46	4
1.7	5.15	6
1.6	4.85	6
1.5	4.55	7
1.4	4.24	9
1.3	3.94	9
1.2	3.64	10
1.1	3.33	10
1.0	3.03	10
0.9	2.73	10
0.8	2.42	10
0.7	2.12	10
0.6	1.82	10
0.5	1.51	10
0.4	1.21	9
0.3	0.91	9
0.2	0.61	9
0.1	0.30	4
0 (water)	0	1
sea water ($\triangle = -1.860$)	3.24	10

prepared as follows: Salts obtained from evaporated sea water were redissolved in sea water and the concentrated sea water thus obtained was filtered. Various dilutions were obtained by adding distilled water to the concentrated sea water. Unmodified sea water was always used as a control.

The dissected mantles were immersed in sea water of different concentration and maintained their luminescence for approximately half an hour. Luminescence persisted longer in dilute than in concentrated sea water. But in all cases luminescence ceased entirely after about an hour. When a mechanical stimulus (a slight rubbing) was applied to a mantle that had already lost its luminescence in the sea water, the luminous organ again illuminated as a response to the stimulus. This irritability was maintained for the longest time in the sea water which was isotonic to 0.6 to 4 per cent NaCl solution. A higher or lower osmotic pressure than this range shortens the time that this irritability will persist.

The results of experiments with NaCl solutions of different concentration were the same, though the absolute value of the persistence of irritability was shorter than in the case of the sea water.

Table 5 is an example out of six series of experiments.

THE ACTION OF IONS ON LUMINESCENCE

It is found by many workers that strong acids, alkalis or salts of heavy metals destroy the luminous power of many luminous organisms. With regard to the action of neutral alkali-metal salts, some stated their exciting action while others found them to be without effect upon bioluminescence. It must be remembered that these experiments were made mostly with luminous insects and bacteria.

Watasenia furnishes good material for studying the action of ions upon bioluminescence. The writer made several series of experiments on this point, which are described in the following three parts.

a. The action of hydrogen and hydroxyl ions. In order to examine the action of H, or OH ions, materials were immersed in the sea water containing HCl or NaOH of a concentration of 1/6400 N to 1/50 N. The reason why HCl and NaOH were chosen for this purpose was that Cl and Na ions are present in large amount in sea water. It was found that H and OH ions first excited luminescence in the squid in proportion to their concentration, and afterwards inhibited the luminescence. H ions destroyed the luminous power, i.e., the irritability to a stimulus, while OH ions had no injurious action upon it, when its concentration was less than 1/50 N.

b. The action of ions of a single compound. In order to study the action of cations and anions separately, chlorides of various metals and Na-salts of various acids were used. Isotonic solution of these

salts were prepared whose osmotic pressure were equal to, or one-half or one-third of that of the sea water ($\Delta = -1.860°C.$). Isomolecular solutions were not used because of a large difference of osmotic pressure between the solutions of univalent and bivalent salts. The concentration of salts in the isotonic solutions was calculated from the formula $\frac{\text{molecular weight}}{\text{molecular freezing point depression}} \times$ freezing point depression of solution.

TABLE 6

The effect of hydrogen and hydroxyl ions on luminescence. Experimental temperature 15.0° to 18.8°C.

CONCENTRATION OF ACID OR ALKALI CONTAINED IN THE SEA WATER	TIME OF PERSISTENCE OF THE LUMINESCENCE	TIME OF PERSISTENCE OF LUMINOUS POWER	
	minutes	*hours*	*minutes*
1/50 N HCl	5		5
1/100 N HCl	5		5
1/200 N HCl	20		20
1/400 N HCl	20	5	
1/800 N HCl	40	5	
1/1600 N HCl	40	6	
1/3200 N HCl	40	5	
Pure sea water	40	6	
1/3200 N NaOH	40	6	
1/1600 N NaOH	40	5	
1/800 N NaOH	40	5	
1/400 N NaOH	40	5	
1/200 N NaOH	40	5	
1/100 N NaOH	20	6	
1/50 N NaOH	5	6	

The molecular freezing point depression of various salts at a fixed osmotic pressure was calculated from the data given in Landolt's "Physico-chemische Tabellen." As for the substances NaI, $NaHCO_3$, NaH_2PO_4, $NaNO_2$, and Na-tartrate, no data being available, the writer measured their molecular freezing point depression by the usual method.

Materials were immersed in these solutions and their luminescence was observed. Tables 7 and 8 are given as examples.

The results with acidic and basic salts, such as NH_4Cl, $NaHCO_3$, NaH_2PO_4 and NaSCN, $NaCH_3CO_2$, Na_2SO_3, Na_2HPO_4, Na-tartrate are not to be taken into account because the solutions of such salts

contain H or OH ions, which act as such upon the materials, though these salts are commonly dealt with by many investigators in a lyotropic series for various physical and physico-chemical properties.

The order of a lyotropic series of ions for physical and chemical properties in acidic solutions is in many cases the reciprocal of that in alkaline solutions. So, in this experiment, the actions of ions in acidic and alkaline solutions were tested, adding 1/600 N HCl and 1/600 N

TABLE 7

The action of cations on luminescence. Experimental temperature 16.4° to 18.6°C.

SALT	CONCENTRATION ($\Delta = -0.93°C.$) IN PER CENT	INTENSITY OF ILLUMINATION	TIME OF PERSISTENCE OF THE ILLUMINATION		TIME OF PERSISTENCE OF THE LUMINOUS POWER	
			hours	*minutes*	*hours*	*minutes*
$LiCl+2H_2O$	2.02	+		40	4	0
NH_4Cl	1.46	−		0		0
NaCl	1.62	+		20	1	20
KCl	2.07	++		20	1	20
RbCl	3.42	−		0		0
CsCl	4.81	++		2		40
$MgCl+6H_2O$	3.58	++	1	20	3	0
$CaCl_2$(sicc.)	2.05	+		20	1	20
$SrCl_2+6H_2O$	5.00	+		20	1	20
$BaCl_2+2H_2O$	4.78	+		2		2
$MnCl_2+4H_2O$	3.62	++		20		20
$FeCl_2+4H_2O$	3.47	++		20		20
$ZnCl_2$	2.40	++		5		5
Sea water	½ dil.	+		20	4	0

Intensity of the luminescence is denoted as follows:
−denotes that the illumination immediately disappears.
+denotes illumination.
++denotes an intensive illumination which is seen from a distance of 80 cm

NaOH respectively to them. But it was found that the results in both cases were the same and not contradictory to each other.

From the results of experiments it may be concluded as follows. The action of alkali- and earth alkali-metal ions on the luminescence is in the following order:

1. The exciting action in the initial stadium

Cations.....Mg > K, Cs > Na, Li, Ca, Sr, Ba > Rb
Anions.....I, NO_3 > SO_4 > Br, Cl, S_2O_3 > NO_2

2. The action concerned in the persistence of the spontaneous illumination

Cations.....Mg, Li > Na, K, Ca, Sr > Cs, Ba, Rb
Anions.....SO_4, S_2O_3 > I, Br, Cl, NO_3 > NO_2

3. The action concerned in the persistence of the luminous power, i.e., irritability against a mechanical stimulus

Cations.....Mg, Li > Na, K, Ca, Sr > Cs > Ba, Rb
Anions.....SO_4 > S_2O_3, I, Br, Cl, NO_3 > NO_2

Thus the Mg ion is the most active.

TABLE 8

The action of anions on luminescence. Experimental temperature 14.8° to 15.8°C.

SALT	CONCENTRATION (Δ = − 0.93°C.) IN PER CENT	INTENSITY OF ILLUMINATION	TIME OF PERSISTENCE OF THE ILLUMINATION		TIME OF PERSISTENCE OF THE LUMINOUS POWER
			hours	*minutes*	*hours*
NaCl	1.62	+		30	1.5
NaBr	2.71	+		30	1
$NaI+2H_2O$	4.95	++		30	1
NaSCN	2.29	++		30	1.5
$NaNO_3$	2.51	++		30	1.5
$NaHCO_3$	2.60	+	1	0	1.5
$NaCH_3CO_2+3H_2O$	3.31	+		5	0.5
$NaNO_2$	1.95	−		0	0
$Na_2SO_4+10H_2O$	7.70	++	1	0	>4
$Na_2S_2O_3+5H_2O$	6.10	+	1	0	1
Na_2SO_3(sicc.)	3.30	−		0	0
$Na_2HPO_4+12H_2O$	8.35	+	1	0	2
$NaH_2PO_4+H_2O$	4.15	−		0	0
Na-tartrate	3.69	+		20	0.5
$MgSO_4+7H_2O$	11.4	++	2	0	>4
Sea water	½ dil.	+	1	30	>4

Ferrous, manganese and zinc ions, the bivalent heavy metal ions used, destroy the luminous power with some initial excitation.

c. The co-action of ions of compounds on luminescence. The co-action of ions contained in the sea water was studied. First of all it was necessary to learn the ionic composition of the sea water. So, before the experiments, the sea water taken from the environment of Watasenia was analyzed. The result is as follows:

Date of collection of the sea water: June 30, 1915. Fair weather.
Specific gravity of the sea water: 1.0255 at 15°C.
Osmotic pressure of the sea water: $\Delta = -1.867$°C.
Salts in 100 cc.:

	grams
NaCl	2.5105
KCl	0.2805
$CaSO_4$	0.1487
$MgSO_4$	0.2033
$MgCl_2$	0.2786

Then the following experiments were made in the next year.

For the sake of convenience, the following composition was assumed as that of the sea water.

	per cent
NaCl	2.51
KCl	0.28
$CaCl_2$	0.12
$MgCl_2$	0.28
$MgSO_4$	0.20
NaBr	0.05

Taking 2.51 per cent NaCl solution as the base, the other components were added in all combinations and the action of these solutions was examined.

The results of these experiments are shown in table 9. From this table it will be seen that Mg, K and Br ions act favorably, but Ca ion unfavorably upon the luminescence of the animal, when their concentrations were equal to those of the sea water.

Next, the effect of varying the ratio of some of the more important combinations of salts was studied.

1. Action of the Na + K system. After examining various ratios of NaCl and KCl concentrations, it was found that the most favorable one was 7 mol NaCl: 1 mol KCl in a rough estimation. This ratio is nearly the same as that of these two substances in the sea water (about 9 mol NaCl : 1 mol KCl). Table 10 serves as an example.

2. Action of the Na + Mg system. Various ratios of NaCl and $MgCl_2$ concentrations were examined and it was found that the higher the concentration of $MgCl_2$, the more favorable was the action of the solution. Table 11 will serve as an example.

3. Action of the Na + K + Mg system. When different amounts of $MgCl_2$ were added to $\frac{1}{2}$ mol NaCl + $\frac{1}{10}$ mol KCl solution, it was found that the favorable action of Mg ion increased in proportion to its amount. Table 12 will serve as an example.

TABLE 9

The co-action of the components of the sea water on luminescence. Experimental temperature 15.4° to 20.0°C.

SOLUTION	TIME OF PERSISTENCE OF THE ILLUMINATION		TIME OF PERSISTENCE OF THE LUMINOUS POWER
	hours	*minutes*	*hours*
NaCl		20	1
NaCl+KCl		20	3
NaCl+$CaCl_2$		10	0.5
NaCl+$MgCl_2$		10	2
NaCl+$MgSO_4$		10	2
NaCl+NaBr		10	2
NaCl+KCl+$CaCl_2$		5	0.5
NaCl+KCl+$MgCl_2$		10	12
NaCl+KCl+$MgSO_4$		20	10
NaCl+KCl+NaBr		20	5
NaCl+$CaCl_2$+$MgCl_2$		10	0.5
NaCl+$CaCl_2$+$MgSO_4$		10	0.5
NaCl+$CaCl_2$+NaBr		10	0.5
NaCl+$MgCl_2$+$MgSO_4$		10	2
NaCl+$MgCl_2$+NaBr		10	3
NaCl+$MgSO_4$+NaBr		10	2
NaCl+KCl+$CaCl_2$+$MgCl_2$		10	12
NaCl+KCl+$CaCl_2$+$MgSO_4$		10	10
NaCl+KCl+$CaCl_2$+NaBr		5	5
NaCl+KCl+$MgCl_2$+$MgSO_4$	2		14
NaCl+KCl+$MgCl_2$+NaBr	2		12
NaCl+KCl+$MgSO_4$+NaBr	2		12
NaCl+$CaCl_2$+$MgCl_2$+$MgSO_4$		10	7
NaCl+$CaCl_2$+$MgCl_2$+NaBr		10	4
NaCl+$CaCl_2$+$MgSO_4$+NaBr		10	4
NaCl+$MgCl_2$+$MgSO_4$+NaBr		10	8
NaCl+KCl+$CaCl_2$+$MgCl_2$+$MgSO_4$		10	10
NaCl+KCl+$CaCl_2$+$MgCl_2$+NaBr		10	10
NaCl+KCl+$CaCl_2$+$MgSO_4$+NaBr		10	10
NaCl+KCl+$MgCl_2$+$MgSO_4$+NaBr	8		14
NaCl+$CaCl_2$+$MgCl_2$+$MgSO_4$+NaBr		5	8
NaC++KCl+$CaCl_2$+$MgCl_2$+$MgSO_4$+NaBr		20	10
Sea water		20	8

4. Action of the Na + Ca system. Various ratio of NaCl and $CaCl_2$ concentrations were examined, and it was found that the solution which contained NaCl and $CaCl_2$ in ratio of 3 : 1–31 : 1 in mol concen-

tration was more favorable than pure NaCl or $CaCl_2$ solution. Table 13 will serve as an example.

TABLE 10

Action of the Na+K *system on luminescence. Experimental temperature 17.5° to 20.0°C.*

CONCENTRATION OF NaCl + KCL IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
		minutes	*hours*
NaCl+KCl	$\frac{1}{2}$ + 0	10	0.5
	$\frac{31}{64}$ + $\frac{1}{64}$	10	0.5
	$\frac{15}{32}$ + $\frac{1}{32}$	30	6.0
	$\frac{7}{16}$ + $\frac{1}{16}$	60	8.0
	$\frac{3}{8}$ + $\frac{1}{8}$	60	6.0
	$\frac{1}{4}$ + $\frac{1}{4}$	30	4.0
	$\frac{1}{8}$ + $\frac{3}{8}$	30	0.5
	0 + $\frac{1}{2}$	30	0.5
Sea water		10	7.0

TABLE 11

Action of the Na+Mg *system. Experimental temperature 24° to 28°C.*

CONCENTRATION OF NaCl + $MgCl_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION		TIME OF PERSISTENCE OF LUMINOUS POWER	
		hours	*minutes*	*hours*	*minutes*
$NaCl+MgCl_2$	$\frac{1}{2}$ + 0		5		20
	$\frac{63}{128}$ + $\frac{1}{128}$		5	1	
	$\frac{31}{64}$ + $\frac{1}{64}$		5	1	
	$\frac{15}{32}$ + $\frac{1}{32}$	1	0	1	
	$\frac{7}{16}$ + $\frac{1}{16}$	1	0	2	
	$\frac{3}{8}$ + $\frac{1}{8}$	1	30	2	
	$\frac{1}{4}$ + $\frac{1}{4}$	1	30	2	
	0 + $\frac{1}{2}$	1	0	1	
Sea water			20	2	

5. Action of the K + Ca system. Various ratio of KCl and $CaCl_2$ concentrations were tested and it was found that the more K ion and the less Ca ion, the more favorably the solution acted. Table 14 will serve as an example.

6. Action of the Mg + Ca system. It was found that the more the concentration of Ca ion, the more unfavorably the solution acted. Table 15 will serve as an example.

TABLE 12

Action of the Na+K+Mg *system. Experimental temperature 24° to 28°C.*

CONCENTRATION OF NaCl + KCl + $MgCl_2$ IN MOL.	TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
	hours	*hours*
NaCl+KCl+$MgCl_2$ $\frac{1}{2} + \frac{1}{10} + 0$	1	2
$\frac{1}{2} + \frac{1}{10} + \frac{1}{128}$	1	2
$\frac{1}{2} + \frac{1}{10} + \frac{1}{64}$	1	2
$\frac{1}{2} + \frac{1}{10} + \frac{1}{32}$	1	2
$\frac{1}{2} + \frac{1}{10} + \frac{1}{16}$	1.5	3
$\frac{1}{2} + \frac{1}{10} + \frac{1}{8}$	2	3
$\frac{1}{2} + \frac{1}{10} + \frac{1}{4}$	2	3
Sea water	1	2

TABLE 13

Action of the Na+Ca *system. Experimental temperature 22.5° to 24.4°C.*

CONCENTRATION OF NaCl + $CaCl_2$ IN MOL.	TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER	
	minutes	*hours*	*minutes*
NaCl+$CaCl_2$ $\frac{1}{2} + 0$	10		30
$\frac{63}{128} + \frac{1}{128}$	10		30
$\frac{31}{64} + \frac{1}{64}$	10	2	0
$\frac{15}{32} + \frac{1}{32}$	10	1	30
$\frac{7}{16} + \frac{1}{16}$	10	1	30
$\frac{3}{8} + \frac{1}{8}$	10	1	30
$\frac{1}{4} + \frac{1}{4}$	20		20
$\frac{1}{8} + \frac{3}{8}$	20		20
$0 + \frac{1}{2}$	25		25
Sea water	25	3	

7. Action of the Na + K + Ca system. To test the action of this system, various amounts of $CaCl_2$ were added to the solution of $\frac{1}{2}$ mol NaCl + $\frac{1}{10}$ mol KCl, and the effects of these solutions were examined. The result was that an addition of $CaCl_2$, even in small amounts, made the solution unfavorable for luminescence. It is, therefore,

possible to assume that the Ca ion content of the sea water (about $\frac{1}{100}$ mol. $CaCl_2$) is unfavorable to the luminescence of Watasenia. Table 16 will serve as an example.

TABLE 14

Action of the K+Ca *system. Experimental temperature 18.2° to 19.6°C.*

CONCENTRATION OF KCl + $CaCl_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
		minutes	*minutes*
$KCl+CaCl_2$	$\frac{1}{2}$ + 0	5	30
	$\frac{5}{12}$ + $\frac{1}{12}$	5	30
	$\frac{2}{6}$ + $\frac{1}{6}$	5	45
	$\frac{1}{4}$ + $\frac{1}{4}$	5	30
	$\frac{1}{6}$ + $\frac{2}{6}$	<5	10
	$\frac{1}{12}$ + $\frac{5}{12}$	<5	10
	0 + $\frac{1}{2}$	<5	10
Sea water		5	>60

TABLE 15

Action of the Mg+Ca *system. Experimental temperature 18.2° to 19.6°C.*

CONCENTRATION OF $MgCl_2$ + $CaCl_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
		minutes	*minutes*
$MgCl_2+CaCl_2$	$\frac{1}{3}$ + 0	10	40
	$\frac{7}{24}$ + $\frac{1}{24}$	20	40
	$\frac{3}{12}$ + $\frac{1}{12}$	20	30
	$\frac{5}{24}$ + $\frac{3}{24}$	20	30
	$\frac{1}{6}$ + $\frac{1}{6}$	20	20
	$\frac{3}{24}$ + $\frac{5}{24}$	10	20
	$\frac{1}{12}$ + $\frac{3}{12}$	5	20
	$\frac{1}{24}$ + $\frac{7}{24}$	5	20
	0 + $\frac{1}{3}$	5	10
Sea water		5	>60

8. Action of the Na + Mg + Ca system. It was found that an addition of $CaCl_2$ to the solution of $\frac{1}{2}$ mol NaCl + $\frac{1}{20}$ mol $MgCl_2$ also made the solution unfavorable for luminescence. Table 17 will serve as an example. The absolute value of the time in this table is shortened due to a high experimental temperature.

9. Action of the Na + K + Mg + Ca system. Lastly it was found that an addition of $CaCl_2$ to the solution of $\frac{1}{2}$ mol NaCl + $\frac{1}{20}$ mol KCl + $\frac{1}{20}$ mol $MgCl_2$ also made the solution unfavorable for the luminescence of Watasenia. Table 18 will serve as an example.

TABLE 16

Action of the Na+K+Ca *system. Experimental temperature 17.0° to 22.2°C.*

CONCENTRATION OF NaCl + KCl + $CaCl_2$ IN MOL.	TIME OF PERSISTENCE OF ILLUMINATION		TIME OF PERSISTENCE OF LUMINOUS POWER
	hours	*minutes*	*hours*
NaCl+KCl+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + 0	1		8
NaCl+KCl+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{320}$	1		4
NaCl+KCl+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{160}$	1		4
NaCl+KCl+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{80}$	1		4
NaCl+KCl+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{40}$	1		4
NaCl+KCl+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{20}$	1		4
Sea water		40	4

TABLE 17

Action of the Na+Mg+Ca *system. Experimental temperature 26 to 28°C.*

CONCENTRATION OF NaCl + $MgCl_2$ + $CaCl_2$ IN MOL.	TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
	minutes	*minutes*
NaCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + 0	40	40
NaCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{314}$	5	40
NaCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{152}$	5	40
NaCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{96}$	5	30
NaCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{48}$	5	20
NaCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{24}$	5	20
NaCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{12}$	5	20
NaCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2}$ + $\frac{1}{20}$ + $\frac{1}{6}$	5	20
Sea water	5	>60

The results mentioned above may be summed up as follows: When all saline components of the sea water (NaCl, KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$ and NaBr) are contained in a solution in the same concentration as in the sea water, the favorable ions for the production of light are in the order of Mg, K and Br, while the most unfavorable one is Ca.

The most favorable ratio of concentration of NaCl and KCl agrees with the ratio in which these two substances are found in sea water.

The favorable action of Mg ion on luminescence increases in proportion to its concentration, quite independently of the other co-existing ions.

Ca ion diminishes the favorable action of K or Mg ion on luminescence.

TABLE 18

Action of the Na+K+Mg+Ca *system. Experimental temperature 26° to 28°C.*

CONCENTRATION OF NaCl + KCl + $MgCl_2$ + $CaCl_2$ IN MOL.	TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
	minutes	*minutes*
NaCl+KCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + 0$	40	60
NaCl+KCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{384}$	5	40
NaCl+KCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{192}$	5	40
NaCl+KCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{96}$	5	40
NaCl+KCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{48}$	5	40
NaCl+KCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{24}$	5	20
NaCl+KCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{12}$	5	20
NaCl+KCl+$MgCl_2$+$CaCl_2$ $\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{6}$	5	20
Sea water	5	>60

SUMMARY

1. Hypotonic sea water lower than $\Delta = -1.8°C.$ (the osmotic pressure of normal sea water) is unfavorable for the maintenance of life of Watasenia. An osmotic pressure lower than $\Delta = -1.5°C.$ shortens the length of its life remarkably. This is the main reason why this animal cannot live in the upper layer of the sea water, which has a very low osmotic pressure.

2. The descriptions of luminescence of the living Watasenia are to be found in the text.

3. The phenomenon of luminescence in this animal is of the intracellular type. The luminous organ of this animal, therefore, is not suitable for extraction of the luminous substances.

4. The phenomenon of luminescence of this animal is evidently due to an oxidation. The disappearance of illumination is observed in the case of absence of oxygen and its reappearance on admission of oxygen.

5. Alcohol, ether and chloroform inhibit this phenomenon in a few minutes, though in the first stage of the process they excite the production of light to a certain degree. The power of illumination is quickly recovered on the removal of the narcotics.

6. The most favorable temperature for the production of light extends from 16° to 31°C. A lower or higher temperature than the above gradually diminishes the illumination and ultimately puts an end to it. This action is reversible. A higher temperature than 49°C. destroys the luminous power owing to heat coagulation.

7. Direct sunlight has no influence upon the luminescence of Watasenia.

8. The most favorable osmotic pressure for the production of light is from 0.2 times to 1.5 times of that of the sea water ($\Delta = -1.8$°C.). Hypotonic or hypertonic pressures lower or higher than these values inhibit the production of light. Initial excitation is, however, seen in the former case but not in the latter.

9. Both hydrogen and hydroxyl ions first excite the production of light, and then inhibit it after a certain time. Hydrogen ions destroy the luminous power while hydroxyl ions have no injurious action upon it, when they are in less concentration than $\frac{1}{50}$ normal.

10. The action of alkali and earth alkali metal ions on the luminescence of the animal is in the following order:

The exciting action in the initial stadium:

Cations.....Mg > K, Cs > Na, Li, Ca, Sr, Ba > Rb
Anions.....I, NO_3 > SO_4 > Br, Cl, S_2O_3 > NO_2

The action concerned in the persistence of the spontaneous illumination:

Cations.....Mg, Li > Na, K, Ca, Sr > Cs, Ba, Rb
Anions.....SO_4, S_2O_3 > I, Br, Cl, NO_3 > NO_2

The action concerned in the persistence of the luminous power:

Cations.....Mg, Li > Na, K, Ca, Sr > Cs > Ba, Rb
Anions.....SO_4 > S_2O_3, I, Br, Cl, NO_3 > NO_2

Among them the Mg ion is the most favorable.

Ferrous, manganese and zinc ion, the bivalent heavy metal ions tested, destroy the luminous power with some initial excitation.

11. When all saline components of the sea water, i.e., NaCl, KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$ and NaBr are contained in a solution in the

same concentration as in the sea water, the favorable ions for the production of light are in the order of Mg, K and Br, while the most unfavorable one is Ca.

The most favorable ratio of concentration of NaCl and KCl agrees with the ratio in which these two substances are found in sea water.

The favorable action of the Mg ion on luminescence increases in proportion to its concentration, quite independently of the other co-existing ions.

The Ca ion diminishes the favorable action of the K or Mg ion on the luminescence of the animal.

In conclusion the writer wishes to express his thanks to Prof. H. Ishikawa for his kind direction and also to Prof. C. Ishikawa for his information about the morphology of Watasenia.

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EXPERIMENTAL MAMMALIAN POLYNEURITIS PRODUCED BY A DEFICIENT DIET

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INTRODUCTION

Progress in medicine is intimately connected with animal experimentation, particularly the reproduction of human diseases in the lower animals. It seems almost superfluous to call attention to the great benefit which modern medical science has derived from the successful reproduction in the common laboratory animals of such infectious diseases as rabies, tuberculosis, trypanosomiasis, syphilis, etc. Discoveries of this nature are usually followed by more rational methods of diagnosis, prevention and treatment. This applies equally well to the diseases of dietary origin, which group includes the so-called deficiency diseases of which beri beri is the best known example. Thus it cannot be questioned that Eijkman's discovery that polyneuritis in fowls could be induced by an exclusive diet of polished rice has very materially contributed to our present conception of the etiology of beri beri and its prevention.

The study of deficiency polyneuritis is also intimately connected with the more recent development of the physiological aspects of nutrition inasmuch as it has been shown that beri beri is due to a deficiency of the diet in a definite substance (antineuritic vitamine), which is essential for normal nutrition. From this standpoint the study of beri beri in animals will undoubtedly shed some light on the physiological function of the antineuritic vitamine. All we know at the present time regarding this function is that a certain minimal amount of this substance must be present in the diet in order to permit normal growth of the young and the maintenance of weight and health of the adult animal and man.

Although birds are very satisfactory for certain work on polyneuritis, their usefulness is obviously limited inasmuch as their anatomy

and physiological behavior differ considerably from those of mammalia. The present investigation aimed, therefore, primari y at the production of deficiency polyneuritis in animals which were more closely related to man and which could be used to better advantage in studies on experimental polyneuritis and the physiological function of the antineuritic vitamine.

Literature referring to mammalian polyneuritis. A perusal of the literature on beri beri and deficiency polyneuritis shows that several investigators have observed symptoms and pathological lesions in mammals, resembling very closely those characteristic of beri beri. However, no systematic attempt seems to have been made to identify this condition as true polyneuritis due to a dietary deficiency.

De Lacerda ('85) reports a study of a disease extensively prevalent among horses and hogs in Brazil. This author calls attention to the great similarity of this disease with beri beri, and also emphasizes the fact that carnivorous animals seemed to be refractory to it.

Hose ('05) very briefly refers to a feeding experiment with three monkeys (*Macacus nemestrinus*) on rice. "One of the monkeys exhibited no special symptoms, but the other two developed some of the characteristic nervous symptoms of beri beri."

Braddon ('07) in his book on the cause and prevention of beri beri graphically describes a disease which he observed in India among horses fed on rice paddy. From his description, he was dealing with a disease very similar to beri beri.

Schaumann ('10) reports experiments on a few rats and dogs which he fed on "denatured" horse meat. The animals lost body weight and after several weeks exhibited paralytic symptoms. The peripheral nerves revealed myelin degeneration of a rather mild degree.

Shiga and Kusama ('11) fed two Japanese monkeys (*Macacus cynomolgus*) exclusively on boiled rice. One of the animals became emaciated and on the 37th day of the experiment developed paretic symptoms in his hind legs. The other monkey died after five months from generalized tuberculosis, without having shown any paralytic symptoms. The necropsy findings of the paretic animal included a slight oedematous condition of the lower parts of the hind legs, increased pericardial fluid, enlargement of the heart and oedema of the posterior lobes of the lungs. Histological examination of the peripheral nerves showed a number of degenerated fibers.

Rommel and Vedder ('15) report a few experiments in which beri beri-like symptoms were observed in pigs fed on rice.

Andrews ('12) produced polyneuritis in seven young puppies which were nursed by Philippine mothers whose infants had died of beri beri.

Osborne and Mendel and McCollum and his collaborators in their work on growth state that they have frequently observed polyneuritis in albino rats which were fed on a deficient diet. We are able to confirm these findings, but we also should like to point out the limited usefulness of rats on account of their small size.

In summing up this review of the literature pertaining to experimental mammalian polyneuritis it is evident that, so far, no method is available by which this disease can be produced with any degree of certainty in the larger mammalia nor have most of the previous investigators clearly demonstrated that the disease which they observed was due solely to a deficiency of the diet in antineuritic vitamine.

EXPERIMENTAL PART

The experiments which will be reported in the following pages were carried out on dogs, cats and albino rats. From preliminary experiments it soon became evident that cats seemed to respond to a deficient diet with the greatest regularity and for this reason most of the work dealt with this species.

In regard to the selection of the food, the dietary habits of the various animals had to be taken into consideration. It stands to reason that a carnivorous animal cannot be fed satisfactorily on vegetable foods to which it is not accustomed. It should therefore be emphasized that the successful prosecution of work of this kind depends mainly on a proper selection of the diet. For instance, adult dogs and cats can be kept for many weeks and months in perfect health on an exclusive diet of beef. This food evidently fulfills all the dietary requirements for this species and was, therefore, adopted in this investigation. Rats, being omnivorous animals, can be maintained for a limited period on meat, but as already pointed out by Watson and Hunter ('06), prolonged meat feeding ultimately leads to various pathological changes.

The question arose as to whether it was possible to destroy the antineuritic substance of beef without otherwise altering the dietary value of this food. As will be seen from the results to be described, meat, even when heated for three hours at 120°C., is still a fairly satisfactory food for cats although its antineuritic power is somewhat reduced by the heating. As exposure of the beef to a relatively high temperature did not seem to yield a product devoid of antineuritic power, it was thought that a combination of heat and alkali might accomplish this purpose. Voegtlin, Sullivan and Myers ('16), for instance, had discovered the fact that the antineuritic substance of "whole" cornmeal is destroyed or inactivated in baking cornbread in the presence of alkali (sodium carbonate). Various other investigators, working on the chemical isolation of the antineuritic vitamine, had also recognized the deleterious action of alkalies on vitamine solutions.

On the basis of these considerations and Schaumann's experiments, the meat was prepared in the following manner: Lean beef[1] is put through a hashing machine. It is then treated under stirring with a 10 per cent solution of sodium carbonate until the mixture is distinctly alkaline to litmus paper. The meat is then heated in an autoclave for three hours at 120°C. After cooling, sufficient dilute hydrochloric acid is added to neutralize the mixture, using litmus paper as an indicator

In order to determine whether heat alone, in the absence of an alkaline reaction, might destroy the physiological activity of the antineuritic vitamine, a number of animals were fed on beef which had been heated for three hours at 120°C without the previous addition of sodium carbonate.

Moreover, the possibility of a combined action of heat and alkali on meat constituents other than the antineuritic vitamine had to be taken into consideration. It was possible, although not very probable, that the symptoms observed in animals fed with alkali-treated meat might have been due to a destruction of certain essential amino acids or the fat-soluble vitamine. The first possibility was met by adding purified casein to the alkali sterilized meat; the second by adding a certain percentage of butter, a food which is known to contain a considerable amount of the fat-soluble vitamine.

The cats, dogs and rats were kept during the whole period of the experiment in small, well ventilated rooms. During the colder season of the year it was found necessary to provide sufficient heat to prevent the occurrence of distemper. The cats and dogs, fed on the deficient diet, seemed extremely susceptible to this disease and we lost a great number of the animals from this cause until the rooms were properly heated. After this precaution was taken there were no further cases of distemper. A sufficient supply of fresh water was provided for all animals.

The following tables, protocols and charts illustrate the main results.

[1] It is important that most of the fat be removed from the meat as otherwise the alkali will cause considerable saponification during the subsequent heating. When this precaution is neglected the animals do not seem to relish the meat and often refuse to eat it altogether.

TABLE 1

Polyneuritis produced in cats by a diet of lean beef, heated for three hours at 120°C. in the presence of sodium carbonate

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
2	30	45	*30th day:* Complete paralysis of legs with intermittent convulsive attacks *31st day:* Died *Necropsy:* No marked, gross pathological findings
5	22	33	*22nd day:* Slight paralysis of legs and convulsive seizure *23rd day:* Received 20 cc. autolyzed yeast filtrate by stomach tube *24th day:* Considerably improved. Has distemper *25th day:* Died. Slight gastro-enteritis Vagus and sciatic: Myelin degeneration
3	27	18	*27th day:* Complete leg paralysis. On being handled, develops clonic spasm. Pupils react very sluggishly to strong light. Given 20 cc. autolyzed yeast filtrate by stomach tube *28th day:* Considerably improved. Sits in normal position. No convulsions. Has distemper *29th day:* No convulsions or marked paralysis, but appears sick *30th day:* Found dead Purulent tracheo-bronchitis. Lungs: passive congestion and oedema. Sciatic: mild myelin degeneration
6	21	38	*21st day:* Marked leg paralysis and clonic convulsions *22nd day:* Signs of distemper noticed. Paralytic symptoms more pronounced *23rd day:* Receives 20 cc. autolyzed yeast filtrate by stomach tube. Most of this is lost by vomiting *24th day:* Slightly improved. 20 cc. yeast filtrate *25th day:* Paralysis much improved. Distemper worse *26th day:* Died. Purulent bronchitis. Pulmonary congestion. Slight gastritis. Sciatic and vagus show some degenerated fiber

TABLE 1—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
7	18	27	*18th day:* Spasticity of hind legs, slight clonic convulsions *20th day:* Condition unchanged. Resp. 28; pulse 198; rect. temp. 38.5°C. *21st day:* Paralysis worse; resp. 33; pulse 186; rect. temp. 38°C. *22nd day:* Severe convulsions; resp. rapid and labored *23rd day:* Found dead. Sciatic: foamy appearance, mild degeneration
8	18	17	*18th day:* Walks with marked incoördination and weakness of hind legs; falls often; resp. 32; pulse 172, rect. temp. 36.5°C. Knee jerks present *20th day:* General appearance about same as last noted. Still shows incoördination. Has convulsions lasting a few seconds *21st day:* Much worse, resp. 70, pulse 68; rect. temp. 34°C. *22nd day:* Found dead. No marked gross pathological findings. Sciatic slightly degenerated
9	14	23	*14th day:* Semi-comatose; unable to walk; knee reflex present. Slight convulsions. Resp. 18; pulse 48, rect. temp. below 32°C. Died at 10 p.m. No marked gross pathological findings except considerable emaciation and slight nephritis. Sciatic: foamy appearance of many fibers
10	24	30	*24th day:* Walks with marked spasticity of legs *25th day:* Found dead *Necropsy:* Purulent bronchitis; otherwise normal. Sciatic shows many fibers with segmentation and foamy-like structure
11	19	32	*19th day:* Unsteady gait, especially marked in hind legs *20th day:* Definitely worse, plaintive cry, cannot take more than a few steps without falling, refuses food *21st day:* Unable to walk, resp. 45, somewhat labored. Given 20 cc. autolyzed yeast filtrate by stomach tube at 11.35 *22nd day:* Much improved, walks about without much difficulty, appetite returned. Seems to relish sterilized meat

TABLE 1—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
11	19	32	*24th day:* Walks about normally, seems to be in fair condition. Given 15 cc. autolyzed yeast filtrate. *25th day:* Not a trace of paresis, seems quite lively. Given 15 cc. autolyzed yeast filtrate *26th day:* Normal appearance, 15 cc. autolyzed yeast filtrate. Eats normally *27th day:* Seems perfectly normal *40th day:* Still in excellent condition. Is fed from now on daily with alkali sterilized meat to which 3 cc. of autolyzed yeast filtrate per kilo of body weight is added. Has gained considerably in body weight *140th day.* Still in excellent condition. Body weight 18 per cent above initial weight. Administration of yeast filtrate is discontinued *211th day:* Refuses food. Seems to be weak. Has no convulsions *215th day:* Staggering gait *216th day:* Found dead. Loss of weight 23 per cent
12	23	8	*23rd day:* Marked incoördination when attempting to walk, falls over as if intoxicated. Clonic convulsions, opisthotonus at times. Refused to eat for last two days. Given 20 cc. autolyzed yeast filtrate. Complete recovery of general health and body weight during the following weeks on a diet of alkali sterilized meat + 1.5 cc. yeast filtrate per kg. body weight *140th day:* Animal in excellent condition
13	20	13	*20th day:* Marked ataxia, from time to time has severe clonic convulsions, during which head and neck are bent forward. Plaintive cries. Resp. 25, pulse 180; rect. temp. 37.3°C. Refuses food. Legs show spasticity. Given 20 cc. yeast vitamine ($FeCl_3$, vitamine no. 3) *21st day:* Can walk, shows but little incoördination. Spasticity of legs has almost completely disappeared. No more convulsions. Given 20 cc. yeast vitamine ($FeCl_3$ vitamine no. 3.)

TABLE 1—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
13	20	13	*22nd day:* Improved, but does not eat; given 20 cc. autolyzed yeast filtrate. Resp. 21; pulse 162; rect. temp. 38.2°C. *23rd day:* Condition slightly worse; one convulsion; given 25 cc. autolyzed yeast filtrate *24thd day:* Seems very much improved; ate some food; does not cry; pulse 134; rect. temp. 38.8°C. Accidentally killed by passing stomach tube into trachea during administration of yeast filtrate
14	24	25	*24th day:* Lost appetite during last week. Shows very definite spastic condition of legs. Arches back and shows typical high stepping gait in hind legs. Pulse 192; rect. temp. 38.6°C. *25th day:* Condition unchanged *26th day:* Symptoms more pronounced. Considerable spasticity; convulsions; given 25 cc. autolyzed yeast filtrate, most of which was vomited up a few minutes later. An additional 15 cc. was given and retained *27th day:* Appears much improved; walks about normally; does not show much spasticity; from this date on animal made rapid recovery, so far as general condition and body weight are concerned, without any change in diet except that cat receives daily 4 cc. autolyzed yeast filtrate per kilo body weight *121st day:* Animal in excellent condition. Gain of body weight 25 per cent
15	18	15	*18th day:* Considerable ataxia when walking. Few severe convulsions lasting a few seconds each are noted. Plaintive cry. Pulse 200; regular; resp. 36; rect. temp. 37.3°C. Paralytic symptoms slightly improved, but after some exercise symptoms gradually grow worse. Marked flow of thick saliva *19th day:* Resp. shallow and irregular; faulty pulse; rect. temp. 38.5°C.; given 15 cc. yeast vitamine ($FeCl_3$ vitamine no. 3, equivalent to 37.5. autolyzed yeast filtrate)

TABLE 1—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
15	18	15	*20th day:* Walks about room with considerable incoordination but is very much improved. Still refuses food. This animal gradually improved after receiving another dose of ferric chloride vitamine, followed by daily administration of 4 cc. autolyzed yeast filtrate per kg. body weight The body weight returned normal and the symptoms entirely disappeared *110th day:* Animal in excellent condition. Escapes
46	53	16	*53rd day:* Hind legs completely paralyzed. Unable to stand. Occasional clonic convulsions lasting for a few seconds. Pupils dilated; react very sluggishly to strong light *54th day:* Found dead *Necropsy finding:* lungs slightly congested. Increase in pericardial fluid. Fatty liver. Kidney pale. Gall bladder distended. Gastro-intestinal tract normal. Sciatic shows extensive myelin degeneration. Vagus only slightly degenerated
47	40	11	*40th day:* Several severe convulsions lasting few seconds. Walks with practically normal gait during intervals. Pulse rate high (cannot be counted) and resp. normal *41st day:* When attempting to walk falls over. Paralysis especially noticeable in hind legs. Fairly well nourished. Pupils enlarged; react very slowly and incompletely to strong sunlight. Pulse 240, regular; resp. 52; shallow, irregular, with tendency to stop during expiration. Plaintive cry. Convulsions can be elicited by handling the animal. Excessive secretion of thick saliva. Used for blood pressure experiment *Necropsy findings:* Increase in clear pericardial fluid. Slight pulmonary congestion. Fatty liver. Spleen, stomach and intestine normal. Kidney pale. Sciatic shows diffuse degeneration. Vagus very slightly degenerated

TABLE 1—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
48	47	29	*47th day:* Animal unable to stand up; paralysis especially marked in hind legs. Pupils enlarged. Pulse very fast. Resp. 45, somewhat labored *48th day:* Condition unchanged. Paralysis more marked. No convulsions *49th day:* Found dead *Necropsy findings:* Lungs normal; heart enlarged; liver fatty; otherwise no significant changes. Sciatic: most fibers show moderate degree of myelin degeneration
51	39	24	*39th day:* Animal, after walking about room for some time, develops slight paralytic symptoms *40th day:* Convulsions have appeared. During intervals between convulsions cat stands with legs spread apart. Hind legs especially weak. When walking, hind legs give way and cat falls on floor. Pupils dilated; resp. 56; irregular, largely abdominal in character. Pulse 240 when lying quietly *41st day:* Complete paralysis of legs. Unable to stand up. Lying on side. Resp. 40, Pulse 240 *42nd day:* Found dead *Necropsy:* Fatty appearance of liver. Other organs practically normal. Sciatic shows foamy appearance and only slight degeneration
52	38	27	*38th day:* Able to stand up but falls down when attempting to walk; paralysis especially pronounced in hind legs. Arched back. Has convulsions at frequent intervals. Resp. 52, regular, largely abdominal; pulse 152, fairly regular *39th day:* Paralysis of hind legs complete. Has tendency to crawl in circle to right. Holds head to right side. Plaintive cry. Palpebral fissure enlarged and pupils dilated. Resp. 72; pulse 232. Given subcutaneously 4 cc. (80 mgm.) yeast vitamine fraction *40th day:* Seems to be slightly improved. Given 30 cc. autolyzed yeast filtrate

TABLE 1—*Concluded*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
52	38	27	*41st day:* Very marked improvement. Animal stands up and walks with pronounced ataxia. No convulsions. Given 20 cc. autolyzed yeast filtrate *42nd day:* Paralytic symptoms further improved. Appetite has returned; 10 cc. autolyzed yeast filtrate given. Treatment from here on omitted and cat finally died 9 days later, after return of symptoms
53	31	32	*31st day:* Swaying gait. Has convulsions from time to time. Does not appear to be in pain. Pulse 84, irregular. Respiration labored at times *32nd day:* Condition unchanged; animal used for blood pressure experiment *Necropsy findings:* Heart normal, very small congested area in right lung. Liver has fatty appearance. Spleen, intestine and kidneys normal in appearance. Sciatic shows advanced myelin degeneration. Vagus, fairly well marked degeneration with foamy appearance
54	34	21	*34th day:* Has severe leg paralysis. Unable to stand. Crawls about. Resp. is periodically very rapid and labored. Pulse 200. Animal used for blood pressure experiment. Sciatic shows very extensive myelin degeneration
55	35	10	*35th day:* Has several convulsions lasting for a few seconds each. Pulse very rapid; impossible to count; resp. normal *36th day:* Condition unchanged *37th day:* Legs show very marked paralysis; resp. irregular and labored. Heart rate very rapid. Pupils enlarged *38th day:* Symptoms still further aggravated. Animal still able to walk. Pulse 250. Resp. irregular, spasmodic at times. Has refused food for several days. Animal used for blood pressure experiment. Sciatic and vagus show moderate myelin degeneration

TABLE 2

Polyneuritis produced in cats by a diet of lean beef, heated for three hours at 120°C. in the presence of sodium carbonate. (Effect of addition of purified casein, or butterfat, or both)

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
100	56	20	(*10 per cent purified casein was added to the meat, after removal from the autoclave*) *56th day:* Considerable weakness in hind legs *57th day:* Weakness in hind legs more marked. Falls over when attempting to walk *60th day:* Symptoms have gradually increased in severity during last 3 days. Marked paralysis of the hind legs. Intermittent convulsions observed. Cat found lying on side in an unconscious condition. Pupils contracted. Corneal reflex practically absent. Respiration abdominal; shallow and irregular. Heart beat 96, regular but very feeble. Pulse in femoral artery cannot be made out. Body cold. Dies *Necropsy:* Emaciated body. Mechanical stimulation of phrenic causes contraction of diaphragm; that of sciatic, contraction of leg muscles. Lungs show small oedematous areas. Heart dilated. Increase in pericardial fluid. Liver congested, fatty appearance. Gall bladder distended. Spleen and kidney congested. Abdominal lymph glands enlarged. Stomach normal except congestion of fundus. Small intestine normal. Large intestine filled with slightly bloody faecal matter. No oedema of subcutaneous tissue. Sciatic, foamy appearance
102	31	19	(*10 per cent purified casein was added to the meat after removal from autoclave*) *31st day:* Walks with arched back, stiffness of hind legs and marked ataxia. Does not appear to be in pain *33rd day:* Has clonic convulsions, with opisthotonus. Rigidity of legs. When walking, ataxia is more marked. Falls over at times on account of hind legs being considerably paralyzed. Rect. temp. 37.5°C. Given 25 cc. melted butter by stomach tube

TABLE 2—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
102	31	19	*34th day:* Paralysis more severe. On attempting to stand, falls on side; still has convulsions. Swaying of anterior part of body when attempting to walk. Pulse 138. Resp. normal. Pupils react very sluggishly to light. Tries to catch mouse but refuses to eat alkali treated beef *35th day:* Completely paralyzed *36th day:* Dead *Necropsy:* No gross pathological findings except that liver presents characteristic "nutmeg" appearance. Sciatic shows mild myelin degeneration
101	33	28	(*10 per cent of casein and 5 per cent of butter fat were added to the meat, after removal from the autoclave*) *33rd day:* Animal has clonic convulsions with opisthotonus and rigidity of legs. Convulsions appear suddenly during which consciousness is not lost. Immediately after the convulsions, which last for a few seconds, the cat gets up and walks about room, showing marked rigidity of legs, arched back, swaying of posterior part of body and high stepping gait. Pupils are enlarged and palpebral fissure is abnormally great (exophthalmus). Cries, but does not seem to be in pain. Tries to catch mouse, but refuses to eat sterilized meat. Sensation to pain in legs not abolished. Receives at noon by stomach tube 18 cc. of a purified yeast vitamine fraction 2 cc. of which had caused the complete recovery of a severely paralyzed pigeon within a few hours. 2 p.m. eats 150 gm. of meat mixture. *34th day:* No convulsions. Walks about room without showing any paralytic symptoms *38th day:* Animal has been free from symptoms until today. Now shows high stepping gait and slight swaying of posterior part of body. No convulsions *44th day:* Has a convulsion immediately after being taken out of cage. Recovers rapidly and attempts to walk, but falls over on left side. Left hind leg seems to be completely paralyzed. When lying quietly has normal appearance, purrs. Respiration 60 abdominal, irregular. 4.00 p.m. Given per os 12 cc, yeast vitamine 123

TABLE 2—*Concluded*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
101	33	28	*45th day:* No convulsions. Walks without showing paralysis. Appears well *54th day:* Shows slight weakness in hind legs. No convulsions. Seems fairly well *72nd day:* Seems weak, but shows also definite ataxia. Plaintive cry *73rd day:* Dead *Necropsy:* Fairly well-nourished body. Nothing abnormal except that ileum is injected throughout. Large intestine contains dry fecal matter. Considerable peritoneal fat. Sciatic, marked myelin degeneration
104	29	22	(*10 per cent casein is added to meat after its removal from the autoclave*) *29th day:* Considerable paralysis of legs. Opisthotonus. Unable to walk. Plaintive cry *32nd day:* Paralytic symptoms have gradually become more aggravated; animal found dead. *Necropsy:* Body in fair state of nutrition. No subcutaneous oedema. Fair amount of subcutaneous fat. Liver shows some fatty change. Kidney, cloudy swelling. No other changes. Sciatic, slight myelin degeneration
105 (see chart 7)	65	9	(*10 per cent casein and 5 per cent butter fat are added to meat after its removal from the autoclave*) *65th day:* Cat has been apparently well until today. 9 a.m. Slight weakness of hind legs. Droopy. 2.30 p.m. Convulsion with opisthotonus. Walks with marked incoördination *66th day:* 9 a.m. Unable to stand or walk. Hind legs completely paralyzed. Uses front legs in attempt to get up. Barely able to crawl. Still has convulsions. Refuses food *68th day:* Condition unchanged *70th day:* Stuporous. Cries as if in pain. Leg paralysis complete *71st day:* Dead *Necropsy:* Heart dilated, flabby. Liver, fatty appearance. Spleen and kidney congested. Spinal cord normal. Cerebrum congested

PROTOCOLS

Dog 3 (see chart 9)

On the 40th day a careful examination of the animal revealed no scorbutic nor neuritic symptoms.

46th day. No definite symptoms except complete loss of appetite.

47th day. Evidence of emaciation. Pulse 144. Rectal temperature 38.5°C. Very excitable. Impossible to determine rate of respiration.

49th day at 10 a.m. Lively and seems to be in good condition. Trembles. Excitable. Temperature 38.1°C.

50th day. Still refuses food but does not appear sick. Temperature 38.8°C. Pulse rate 108.

51st day. Lively, no change. Temperature 38.6°. Pulse rate 124, irregular.

52nd day. Eats well. Temperature 39°. Pulse rate 90, irregular. Respiration 20.

68th day. Has been eating well. Is lively and of normal appearance.

70th day. Appears very gaunt. Shivers. Unable to hold up his head. Gait spastic. On taking a few steps the hind legs stiffen and are dragged. Cannot stand up more than 2 minutes. Temperature 37.6°C. Pulse 96. Used for blood pressure experiment.

Dog 5 (see chart 9)

The first symptoms were observed on the 46th day of the experiment, when the dog began to refuse his food.

48th day. Pulse 120. Respiration 16. Rectal temperature 38.9°C.

50th day. Dog lively and somewhat excited. Pulse 168, slightly irregular. Rectal temperature 38.9°C. After the dog becomes more quiet pulse drops to 140.

51st day. Pulse 105. Temperature 39°C. Still refuses food but seems quite lively.

52nd day. Eats a small amount of food. Seems to be in good condition. Pulse 140. Respiration 20. Temperature 38.3°C.

53rd day. Appetite and general appearance good. Pulse 114. Respiration 15. Temperature 39°C.

54th day. Pulse 104. Irregular. Respiration 16. Temperature 38.7°C. From this time to the 70th day, dog was apparently in good condition.

70th day, 9.30 a.m. Definite symptoms consisting of marked spasticity of hind legs, increased patellar reflexes. Pulse 68, very irregular. Respiration 12. Temperature 38°C. On very slight exertion the pulse increases to 150 and the respiration to 18.

10.30 a.m. A mild convulsion. Dog used for kymograph experiment.

Dog 6. Male. 10.9 kilos (see chart 9)

Up to 45th day dog was in generally good condition. On this day at 9.00 a.m. the dog was found lying on floor. Able to stand up. Walks a few steps with staggering gait and falls down again. Appears gaunt. Conjunctivitis. The gums are slightly congested but show no hemorrhages. Knee jerks are exaggerated. Refuses food.

12.30 p.m. Unable to stand. Given 50 cc. autolyzed yeast filtrate per stomach tube. Some of this yeast product was lost by vomiting (probably not more than 20 cc.)

4.30 p.m. Condition unchanged.

46th day 10.30 a.m. Appears greatly improved. Walks almost normally, but becomes easily fatigued and falls on floor. Drinks water eagerly. Pulse 122, irregular. Respiration 10. Rectal temperature 39°C.

11.30 a.m. Runs about normally.

11.50 a.m. After exercise, pulse 140; respiration 12.

1.05 p.m. Dog has been quiet for some time. Pulse 112. Respiration 8. Temperature 38.8°C.

48th day, 9 a.m. Walks almost normally. After walking about, pulse 150, less irregular than previously. Respiration 11. Temperature 38.5°C. Still refuses food.

49th day. Gait is practically normal. Pulse 175. Respiration 11. Temperature 38.7°C.

50th day. No marked change. Urine: considerable albumen, hyaline and granular casts, bile pigments, a few pus and blood cells. Fehling, negative.

51st day. Still refuses sterilized meat. No change in condition. Given 400 cc. milk + 30 cc. autolyzed yeast filtrate. Dog drinks this mixture with eagerness.

52nd day. General condition good. Has eaten some sterilized meat. Given 400 cc. milk + 30 cc. yeast filtrate. The respiration has increased to 15; the pulse is 152. Temperature 38.8°C.

53rd day. Given 400 cc. milk + 30 cc. yeast filtrate, which is taken at once. The conjunctivitis which has been treated with boric acid has practically cleared up.

54th day. Eats 475 cc. milk + 30 cc. yeast filtrate.

55th day. 475 cc. milk.

76th day. Diet changed to 400 gm. sterilized meat + orange juice. This food is eaten very well by dog.

102nd day. Dog has been in fairly good condition until a few days ago. No paralytic symptoms were observed and appetite was excellent. However, the dog developed a skin disease (mange) and gradually lost body weight. Today the animalwas found dead. Necropsy reveals nothing abnormal except a slightly inflamed condition of gastro-intestinal tract. Sciatic nerve: myelin degeneration.

Dog 7 (see chart 10)

This animal during the first part of the experiment ate the alkali-treated meat with great avidity.

On the 31st day the animal refused its food and appeared to be not as well as usual, but no paralytic symptoms were observed.

32nd day. Appears sick. Refuses food. Slight tremor of body. Falls down on running or turning quickly. Knee reflex seems to be exaggerated. Some stiffness of muscles of neck and hindlegs. Gums normal. Examination of urine shows strongly acid reaction; considerable heat-coagulable protein; bile pigment; a few granular casts. Rectal temperature 37.8°C. Respiration 20. Pulse 126, very irregular.

34th day. Condition slightly worse. Gait more unsteady. Falls over easily. Pulse 80, irregular. Respiration 20. Rectal temperature 38.3°C. Vomits.

35th day. Condition of dog becoming progressively worse. Can still walk but falls easily. Reflexes exaggerated. Pulse 130, very irregular. Respiration 20. Rectal temperature 37.6°C. Used for blood pressure experiment.

Dog 10 (see chart 10)

This animal ate well and was in good condition during the first 28 days of the experiment. From this time on the appetite decreased and on the 42nd day the dog showed, for the first time, definite signs of paresis in hind legs. On the 45th day the dog was found dead.

Necropsy: Emaciated body. No scorbutic changes. Lungs normal; heart dilated; liver, spleen and kidneys normal; stomach shows gastritis, which is largely confined to fundus region. Duodenum shows mild inflammation. Sciatic shows slight myelin degeneration. The urine of this animal was examined the day before death occurred, with the following results: specific gravity 1018; coagulable protein, trace; a few casts; Fehling, negative; acetone and diacetic, negative.

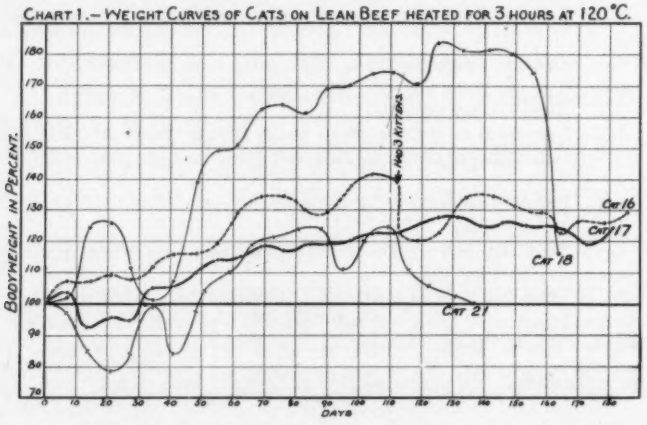

Chart 1. Illustrates the fact that adult cats can be kept for a considerable period in perfect condition, when fed exclusively on lean beef heated for 3 hours at 120°C. (in the absence of free alkali). Cats 16 and 17 lived on this diet for over 180 days and were in every respect normal at the end of this period. Cat 18 died suddenly on the 164th day of the experiment, without having shown any paralytic symptoms. The cause of death in this animal could not be established but polyneuritis was not responsible for the death, as the peripheral nerves did not exhibit any myelin degeneration. Cat 16 on the 114th day of the experiment gave birth to 3 kittens which died 2 days later. The sciatic nerves of these kittens did not show any myelin degeneration. Cat 21 died on the 139th day of distemper. These experiments show that the autoclaving of the meat, *in the absence of an alkaline reaction*, does not completely destroy the antineuritic vitamine contained in fresh beef.

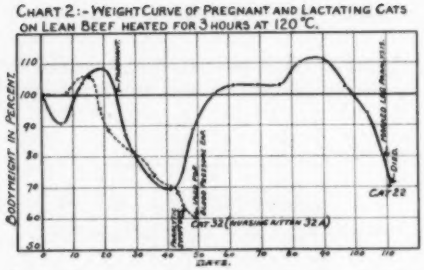

Chart 2. Shows that pregnant and lactating animals are apt to develop polyneuritis on a diet of beef which was heated for 3 hours at 120°C. Pregnancy and lactation, therefore, render the animals more susceptible to polyneuritis, an observation which is in complete agreement with the observations made on beri beri in the human. Here also it was found that pregnancy and lactation favor the appearance of the disease.

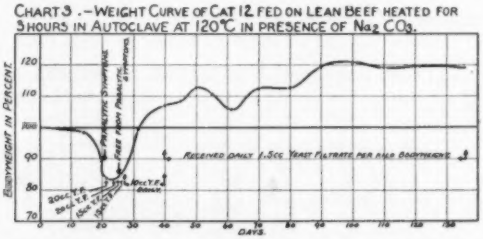

Chart 3. Illustrates the loss of weight preceding the appearance of polyneuritis on a diet of alkali-treated meat. The oral administration of autolyzed yeast relieved the symptoms, and the continued daily administration of a small amount (1.5 cc.) of autolyzed yeast filtrate prevented the reappearance of the symptoms. The animal not only recovered its original body weight but surpassed it by 20 per cent. The cat was in perfect condition on the 137th day when the experiment was discontinued.

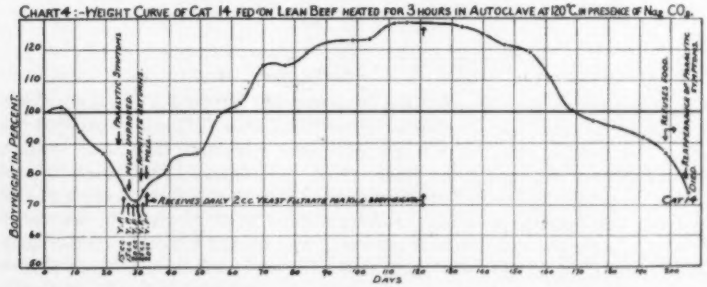

Chart 4. Shows the results of an experiment similar to that illustrated by Chart 3. It also shows that discontinuation of the autolyzed yeast filtrate is followed by a recurrence of polyneuritis.

Weight curve of cat 102 on lean beef heated for 3 hours at 120°C. in presence of Na_2CO_3. After cooling 10 per cent purified casein is added to meat.

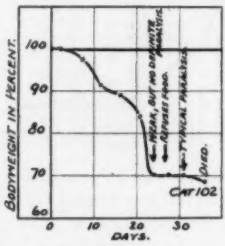

Chart 5. Illustrates the fact that the disease produced by exclusive feeding on alkali-treated beef is not due to a deficiency of the diet in certain essential amino acids, as the addition of 10 per cent purified casein to this meat does not prevent the appearance of paralytic symptoms.

Weight curve and food consumption of cat 101, fed on lean beef heated for 3 hours at 120°C. in presence of Na_2CO_3; 10 per cent purified casein added after heating.

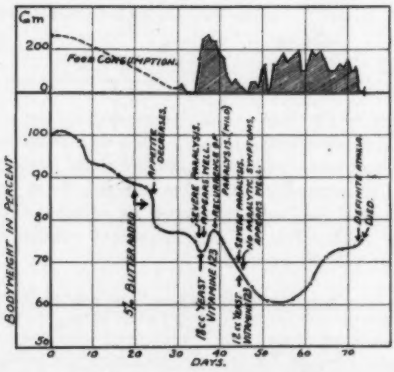

Chart 6. Shows that the addition of casein and butter fat to alkali-treated beef does not prevent the appearance of the symptoms. This is in marked contrast to the striking effect of a single comparatively small dose of an antineuritic preparation obtained from yeast; 18 cc. (equivalent to 360 mgm.) of this preparation promptly caused the complete disappearance of severe paralytic symptoms, the animal appearing perfectly normal 20 hours after the treatment. A few days later the symptoms reappeared, and on the 10th day following the first treatment the animal was again severely paralyzed; 12 cc. (equivalent to 240 mgm.) again relieved the symptoms for several days. Note influence of treatment on food consumption.

Weight curve and food consumption of cat 105 fed on lean beef heated for 3 hours at 120°C. in the presence of Na_2CO_3. After cooling 10 per cent casein and 5 per cent butter are added.

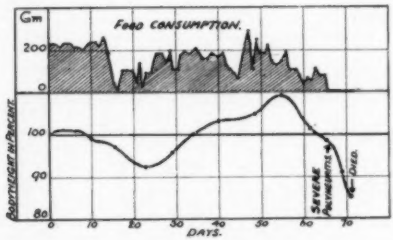

Chart 7. Shows that the addition of butter and purified casein to the alkali-treated meat does not prevent the appearance of polyneuritic symptoms. It happens that in this animal the incubation period is considerably longer than in cats fed on meat without the addition of butter and casein.

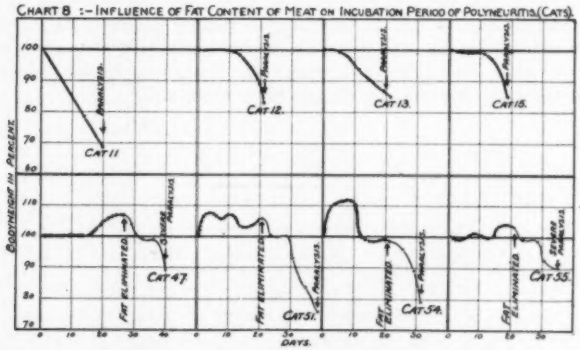

Chart 8. The upper series of animals (no. 11 to 15) was fed on beef which had been freed from fat as much as possible previous to the heating for 3 hours at 120°C. in the presence of Na_2CO_3. The second series (no. 47, 51, 54 and 55) received during a preliminary period (indicated by broken line) beef which was treated in exactly the same manner as mentioned above, with the exception that the fat had not been removed. Note the fact that on this diet the animals do not lose weight. As soon as the fat was eliminated the animals also began to lose weight and to develop polyneuritic symptoms. These results justify the conclusion that the presence of a considerable amount of fat renders the anti-neuritic vitamine of meat more resistant to the combined action of a high temperature (120°C.) and alkali.

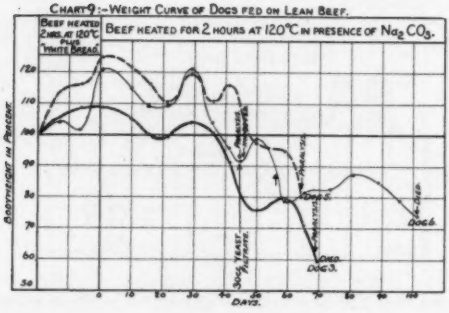

Chart 9. Illustrates the loss of body weight of dogs fed on alkali-treated meat. During a preliminary period of 20 days the animals were fed on a mixture of beef, which had been heated for 2 hours *without the previous addition of alkali*, and "white" bread. This food mixture caused an increase in body weight. On changing the diet to beef which had been heated with alkali, the animals gradually lost weight and developed typical polyneuritis. Dog 6 was severely paralyzed on the 45th day and received about 30 cc. autolyzed yeast filtrate by stomach tube. The following day the symptoms had almost completely disappeared. For further details see protocols.

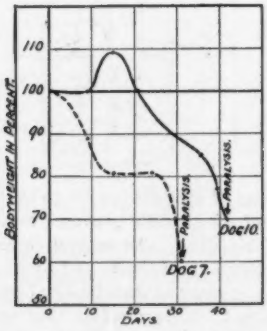

Chart 10. Weight curve of dogs fed on lean beef heated for 2 hours at 120°C. in the presence of Na_2CO_3.

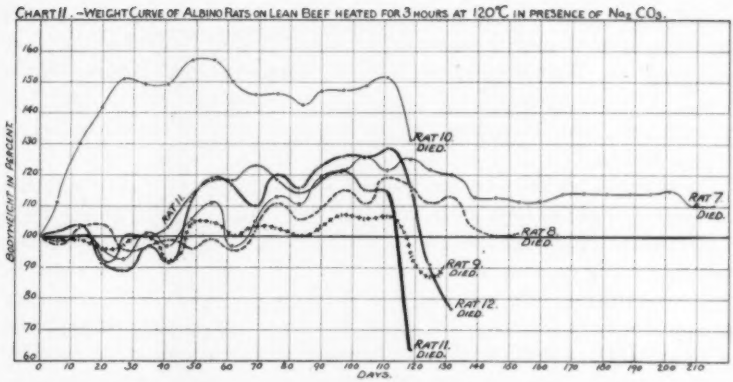

Chart 11. Shows that albino rats are much more resistant to polyneuritis than either cats or dogs. The meat fed these rats was prepared in exactly the same manner as the meat which was used for the feeding of the dogs and cats. Note the increase in body weight up to the 110th day. Rat 10 was a young animal weighing 110 gm. a tthe beginning of the experiment. This rat showed very considerable growth during the first 25 days. No symptoms characteristic of polyneuritis were observed in these animals. About the 110th day the weather became very warm (July) and the animals developed diarrhoea, which very probably was responsible for the sudden death of four of the animals.

DISCUSSION OF RESULTS

One of the most interesting points brought out by this research is the great difference in susceptibility to polyneuritis between the various species of animals. According to Fraser and Stanton ('09) the shortest incubation period for beri beri in man on a diet of highly milled rice is at least eighty-seven days. Pigeons fed on the same rice develop the disease in an average of twenty-one days, chickens in twenty-eight days. In our cats, fed on alkali-treated meat, the earliest polyneuritic symptoms were noticed on the eighteenth day. The dogs usually required about a month to six weeks for the appearance of the symptoms; and the rats (adult) lived for at least one hundred and ten days on the same deficient diet (alkali-treated beef) without showing the faintest indication of polyneuritis. One rat even lived for two hundred days, apparently in perfect health. The cats responded to the deficient diet with the greatest regularity, all of them (28) developing the disease. For unknown reasons, two of the dogs were refractory, although typical polyneuritis was observed in other dogs.

Symptomatology. As a rule the animals (dogs and cats) appeared perfectly normal and devoured the alkali-treated meat for the first week or two. Sooner or later, however, their appetite became capricious. A very marked and persistent constipation set in and the animals lost their liveliness and appeared drowsy. They then began to lose weight.

There was a great individual difference in the manner in which the paralytic symptoms made their appearance. An animal would seem to be normal until it was taken out of the cage, when it would suddenly develop a tonic convulsion which usually lasted for only a few seconds. These convulsive seizures would return many times until gradually they gave way to a progressive paralysis of the legs and the animal was no longer able to walk or stand. This condition was soon followed by death. In other cases the paralysis first revealed itself by slight weakness. The animal tired easily and refused to walk. This was usually followed in a day or two by incoördination of the gait. Often spastic contraction of certain muscles of the hindlegs resulted in a very typical spastic gait, simulating the "steppage" gait of multiple neuritis in the human. In some cats the back showed a persistent arching, resembling the position caused by fright. Anaesthesia was rarely present, except in the very advanced stages of the disease. The animals at times seemed to be in pain, as evidenced by the plaintive cries characteristic of cats.

The reflex excitability was often exaggerated. Stimulation of the cut vagus or sciatic caused qualitatively normal reactions (see fig. 2) even in animals with far advanced paralysis. Electric stimulation of the cervical sympathetic caused prompt dilatation of the pupil.

The respiration was usually slowed and very often was irregular (see fig. 1).

The pulse was considerably accelerated during the acute stage; in some cases so rapid that it could not be counted. Irregularities were frequent. Slight muscular exertion, as walking up stairs, caused tachycardia. The blood pressure was practically normal. The pupils were often enlarged and reacted sluggishly to light.

The urine was greatly diminished in volume and gave a strong test for indican, a trace of coagulable protein and bile pigments. Acetone, diacetic acid and sugar were absent. Casts were present in small numbers.

There was a conspicuous absence of oedema in our animals, a symptom which is characteristic of so-called wet beri beri in the human.

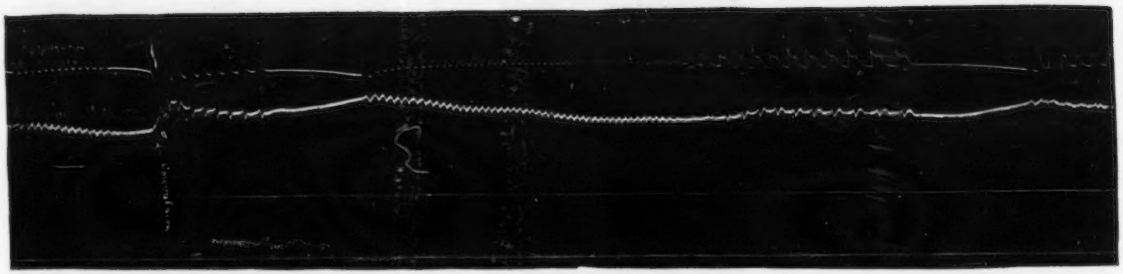

Fig. 1. Cat 47. Light chloretone anaesthesia. Respiratory tracing obtained from pleural cannula. Upstroke indicates inspiration. Blood pressure measured in carotid artery. This tracing shows the marked irregularities in the respiration of a cat with severe polyneuritis. The fluctuations in the blood pressure follow very closely the changes in respiration. Note effect of a convulsion on blood pressure and respiration. The respiration is temporarily very much increased and the blood pressure raised. A short period of apnoea precedes the convulsion.

The disease never caused fever; on the contrary the body temperature fell considerably toward the end. Even in the earlier stages of the disease the heat regulation was upset, as evidenced by a subnormal body temperature resulting from exposure of the animal to a cold room in the winter time. We were unable to explain this disturbance of heat regulation; it may possibly have its origin in an alteration of the nervous mechanism which controls the body temperature.

Death usually occurred several days after the first appearance of the paralytic symptoms. Exceptionally, animals might die during the night, although no definite paralytic symptoms were observed during the previous days.

Pathology. Gross. The gross changes were not very marked. There may be mentioned: fatty appearance of the liver, congestion of the

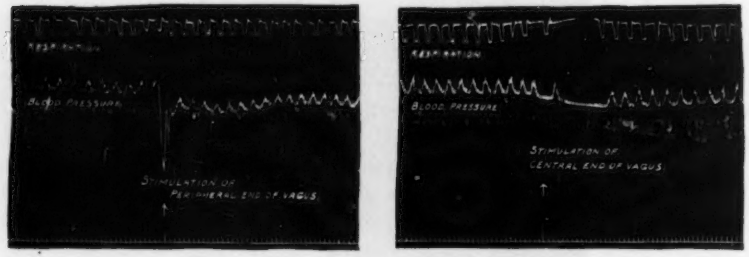

Fig. 2. Dog (no. 7) with severe polyneuritis. Shows effect of stimulation of central and peripheral end of cut vagus. Note normal character of the reaction.

kidney, inflammation of the upper portion of the intestine, dilatation of the heart and occasionally an increase in the pericardial fluid. With the exception of two of the dogs, no evidence of scurvy was found in any of the animals.

Histological. In almost every instance the sciatic nerves were examined by the Marchi method for evidence of myelin degeneration. In a considerable number the vagi were also examined. Fairly complete histological examinations were made of practically all the important organs of several of the cats. Most of the tissues were fixed with formalin, embedded in colloidin and stained with haematoxylin and eosin. Sections were made from several levels of the spinal cord which were treated by the Marchi method, embedded in paraffin and mounted in chloroform balsam.

The most striking changes were found in the nervous system. The teased sciatic nerves of practically all the animals which developed symptoms, showed myelin degeneration varying from a mild type, characterized by a foamy appearance of the myelin and a swelling at the nodes of Ranvier, to a typical, well-marked degenerative process, involving many fibers, similar to that seen in pigeons with polyneuritis gallinarium. The few spinal cords studied showed some degenerated fibers at all levels, the changes being quite similar to those described by Vedder and Clark ('12) in the case of pigeons. As to location they were somewhat scattered, being found in the posterior, lateral and anterior columns. The amount of change found in the nerves and cord did not always correspond to the severity of the symptoms in the animals.

The changes found in the parenchymatous organs may be summarized as follows:

Heart: The transverse striations are for the most part absent, or only faintly defined. There is considerable fragmentation of the sarcoplasm. It is difficult to determine whether this is due to agonal or post-mortem changes, or to those due to fixing, but it seems that a more or less acute parenchymatous degenerative process must have been present. Fatty degeneration was present only to a slight degree.

Lungs: Endarteritis is quite marked in several cases. The interalveolar septa are thickened. The capillaries are thickened. The capillaries are distended and phagocytic cells are quite numerous in the septa and to some extent in the alveolar lumina. Pigmentation is not marked.

Small intestine: The mucosa shows quite extensive changes. The villi are largely gone. Some round-celled infiltration is present in the base of the mucosa and extends into the submucosa.

Liver: The central vein and its capillaries are distended with blood. The liver cells show numerous small vacuoles, as well as a few large ones, and, besides, a highly granular condition of the cytoplasm.

Pancreas: No definite changes were found with the method of staining employed.

Spleen: There is a marked increase in the connective tissue elements in some cases amounting almost to a true interstitial splenitis. There is a slight amount of colloid degeneration. There are more blood cells in the Malpighian corpuscles than normal and pigmented tissue cells are numerous.

Kidney: The capsule shows no thickening. The glomeruli are for the most part nearly normal, except that the capillaries are somewhat distended. A few of the glomeruli are shrunken and show colloid degeneration, with some round celled infiltration in the vicinity. There are a few small capillary haemorrhages. The epithelial cells of the

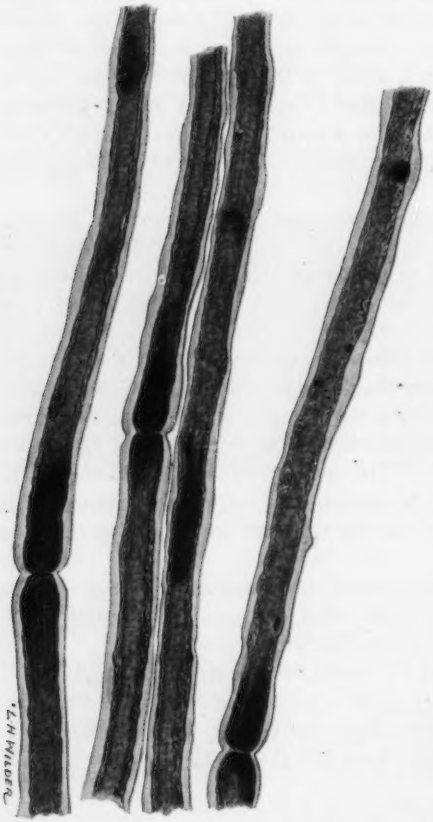

Fig. 3

Fig. 4

Fig. 3. Cat 22. Sciatic nerve, teased preparation, Marchi method. Shows marked myelin degeneration. See chart 2.

Fig. 4. Cat 53. Sciatic nerve, teased preparation, Marchi method. Shows fairly well marked myelin degeneration.

convoluted tubules show considerable vacuolization and more than the usual amount of granulation of the cytoplasm. The nuclei, for the most part, stain fairly well. In some instances the lumina of the tubules are completely obliterated, the cytoplasm of the cells forming a more or less homogeneous mass of granular material, with the poorly staining nuclei scattered about.

These pathological changes are somewhat similar to, though less extensive than those described by Sundwall ('17) in connection with studies in tissue alterations in malnutrition and in pellagra.

Treatment. When the disease had not advanced too far, the animals could be relieved of their symptoms by means of an active preparation of the antineuritic substance. As will be seen from the protocols, the administration of a single dose of autolyzed yeast filtrate to an animal with severe paralysis caused complete recovery, often within twelve hours. The same result was obtained also after the administration of a single dose of a purified yeast preparation, which had been freed from amino acids, purine bases and other impurities (yeast vitamine 123, chart 6). The improvement, following the administration of antineuritic preparations to severely paralyzed cats, was just as striking as that obtained in pigeons. There was prompt disappearance of all the clinical signs such as paralysis, loss of appetite, high and irregular pulse rate, labored respiration, etc. If the treatment was not continued the symptoms returned after a few days. The continuous daily administration of even a very small dose of autolyzed yeast filtrate resulted in the recovery of lost body weight and prevented the reappearance of polyneuritis.

General considerations. Numerous writers have called attention to the fact that beri beri in the human being is associated with a diet rich in carbohydrates. The disease is especially prevalent among eastern peoples who live on a more or less exclusive diet of white rice. The observations of Little concerning beri beri in Newfoundland seem also to confirm this view, as he attributes the disease to a diet composed of white bread, molasses and fish. Funk ('14) on the basis of feeding experiments on pigeons, tried to establish a relation between carbohydrate metabolism and the antineuritic substance. This author believes that the antineuritic vitamine is in some way concerned with the metabolism of carbohydrates, as he found that an increase in the carbohydrate component of the diet caused a more rapid appearance of avian polyneuritis. This conclusion is also confirmed by the extensive experiments of Braddon and Cooper ('14) who state

that the amount of antineuritic substance required by the organism increases with the quantity of carbohydrate metabolized. For the maintenance of health the intake of active substance must, therefore, be adjusted so as to stand in some quantitative relation to the amount of carbohydrate included in the diet.

On superficial consideration, these statements seem to be contradicted by the fact that the present authors were able to induce typical

Fig. 5

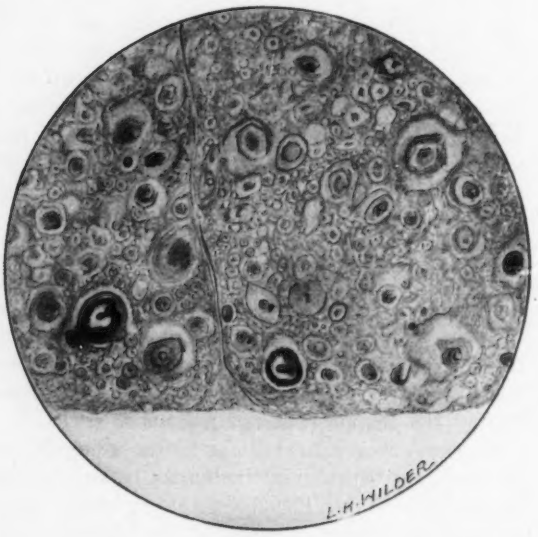

Fig. 6

Fig. 5. Cat 51. Sciatic nerve, teased preparation, Marchi method. *A*, shows early stage of myelin degeneration—foamy appearance. *B*, similar preparation from normal cat.

Fig. 6. Cat 4. Cross section of spinal cord (lumbar region), lateral column; Marchi method. Shows degenerated fibers.

polyneuritis by means of an exclusive meat diet, a food which is rich in protein and very poor in carbohydrate. It should be remembered, however, that the intermediate metabolism of protein may lead to the formation of large amounts of carbohydrate. For instance, Reilly Nolan and Lusk ('98) fed a dog suffering from phlorizin diabetes 500

Fig. 7. Dog. 6. *A*, sciatic nerve, teased preparation, Marchi method, showing myelin degeneration. *B*, fiber from sciatic of normal dog.

grams of meat and calculated that the sugar production from meat amounted to 58 per cent by weight of the meat protein metabolized. Our observations, therefore, merely supplement the view, expressed by Funk, Braddon and Cooper, that a high protein diet may also lead to an increase in carbohydrate metabolism which may require the presence of a minimum quantity of antineuritic substance.

From the standpoint of dietetics, the results of this investigation emphasize the great stability of the antineuritic substance of meat after a relatively long exposure of this food to high temperatures. Beef can be heated for three hours to 120°C. without its completely losing its antineuritic properties (see chart 1). It is probable that the acid products (lactic acid) of beef are partly responsible for this great heat resistance of the antineuritic substance, as it was shown in this laboratory and elsewhere that an acid reaction tends to preserve its physiological activity. In the preparation of beef for human consumption, this food is rarely exposed throughout to a temperature exceeding 100°C. On the other hand, this research demonstrates that a combination of high temperature and an alkaline reaction leads to the complete destruction of the antineuritic properties of beef. Even in the presence of an alkaline reaction, it is necessary to heat the meat for a considerable time at a temperature above 100°C. Short exposure to 100°C. does not seem to bring about a loss of antineuritic power, as evidenced by some negative experiments not reported in this paper.

In conclusion, it should be pointed out that the present tendency toward alterations of the dietary, as brought about by the war, may very well lead to the occurrence of isolated cases of deficiency diseases in this country and abroad. It is very likely that the early stages of beri beri might easily escape recognition, as the symptoms are not specific for this disease only. This statement is supported by the fact that the prevalence of beri beri among the fishermen of Newfoundland had evidently escaped recognition until Little published his report describing the widespread existence of beri beri in this locality. The history of pellagra in the United States also furnishes evidence to the effect that an unfamiliar disease may exist for years before its presence is recognized.

CONCLUSIONS

1. Polyneuritis has been produced in cats and dogs as the result of an exclusive dietary of lean beef which was heated for three hours at 120°C. in the presence of alkali (sodium carbonate). Proof of this statement is furnished by the symptomatology, treatment and pathology of the disease noted, which are essentially those characteristic of beri beri.

a. Symptoms: The following symptoms were observed in these animals: Diminution of appetite, constipation, loss of body weight, weakness and sometimes drowsiness, followed by paralytic symptoms, tonic convulsions, spasticity of certain groups of muscles and disturbances of the circulation and respiration.

b. Treatment: The oral administration of active preparations of the antineuritic substance of yeast to paralyzed animals is followed promptly by the disappearance of the symptoms; and the continued administration of these preparations prevents the recurrence of the disease.

c. Pathological changes: Certain histopathological changes especially the changes involving the nervous system are described. Animals showing severe paralysis exhibit no qualitative changes in the reaction of various nerves to electric stimulation.

2. The disease is due to a deficiency of the diet in antineuritic substance and not to a deficiency in the other essential dietary components (amino acids, fat-soluble vitamine, etc.).

3. Exposure of the beef for three hours to a temperature of 120°C., without the previous addition of alkali, does not completely destroy the antineuritic power of this food. It is therefore concluded that the ordinary preparation of meat for human consumption does not lessen its food value in this respect.

4. The various species of animals show a considerable difference in their susceptibility to polyneuritis, as evidenced by the different length of time which is necessary to induce the disease by the same deficient diet. Cats respond to the deficient diet with the greatest regularity and are, therefore, best adapted for physiological studies of the function of the antineuritic substance.

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